

**COMPOSITIONS AND METHODS FOR THE TREATMENT OF IMMUNE RELATED DISEASES**Field of the Invention

5       The present invention relates to compositions and methods useful for the diagnosis and treatment of immune related diseases.

Background of the Invention

10      Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a reaction to self, or as a combination of these.

15      Though the genesis of these diseases often involves multistep pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

20      Many immune related diseases are known and have been extensively studied. Such diseases include immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc.

25      T lymphocytes (T cells) are an important component of a mammalian immune response. T cells recognize antigens which are associated with a self-molecule encoded by genes within the major histocompatibility complex (MHC). The antigen may be displayed together with MHC molecules on the surface of antigen presenting cells, virus infected cells, cancer cells, grafts, etc. The T cell system eliminates these altered cells which pose a health threat to the host mammal. T cells include helper T cells and cytotoxic T cells. Helper T cells proliferate extensively following recognition of an antigen -MHC complex on an antigen presenting cell. Helper T cells also secrete a variety of cytokines, i.e., lymphokines, which play a central role in the activation of B cells, cytotoxic T cells and a variety of other cells which participate in the immune response.

30      Immune related diseases could be treated by suppressing the immune response. Using neutralizing antibodies that inhibit molecules having immune stimulatory activity would be beneficial in the treatment of immune-mediated and inflammatory diseases. Molecules which inhibit the immune response can be utilized (proteins directly or via the use of antibody agonists) to inhibit the immune response and thus ameliorate immune related disease.

35      CD4+ T cells are known to be important regulators of inflammation. Herein, CD4+ T cells were activated and the profile of genes differentially expressed upon activation was analyzed. As such, the activation specific genes may be potential therapeutic targets. *In vivo* co-stimulation is necessary for a productive immune proliferative response. The list of costimulatory molecules is quite extensive and it is still unclear just which co-stimulatory molecules play critical roles in different types and stages of

inflammation. In this application the focus is on genes which are specifically upregulated or downregulated by stimulation with anti-CD3/ICAM, or anti-CD3/anti-CD28 and may be useful in targeting inflammatory processes which are associated with these different molecules.

Despite the above identified advances in T cell research, there is a great need for additional diagnostic and therapeutic agents capable of detecting the presence of a T cell mediated disorders in a mammal and for effectively reducing these disorders. Accordingly, it is an objective of the present invention to identify polypeptides that are overexpressed in activated T cells as compared to resting T cells, and to use those polypeptides, and their encoding nucleic acids, to produce compositions of matter useful in the therapeutic treatment and diagnostic detection of T cell mediated disorders in mammals.

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#### Summary of the Invention

##### A. Embodiments

The present invention concerns compositions and methods useful for the diagnosis and treatment of immune related disease in mammals, including humans. The present invention is based on the identification of proteins (including agonist and antagonist antibodies) which are a result of stimulation of the immune response in mammals. Immune related diseases can be treated by suppressing or enhancing the immune response. Molecules that enhance the immune response stimulate or potentiate the immune response to an antigen. Molecules which stimulate the immune response can be used therapeutically where enhancement of the immune response would be beneficial. Alternatively, molecules that suppress the immune response attenuate or reduce the immune response to an antigen (e.g., neutralizing antibodies) can be used therapeutically where attenuation of the immune response would be beneficial (e.g., inflammation). Accordingly, the PRO polypeptides, agonists and antagonists thereof are also useful to prepare medicines and medicaments for the treatment of immune-related and inflammatory diseases. In a specific aspect, such medicines and medicaments comprise a therapeutically effective amount of a PRO polypeptide, agonist or antagonist thereof with a pharmaceutically acceptable carrier. Preferably, the admixture is sterile.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprises contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native sequence PRO polypeptide. In a specific aspect, the PRO agonist or antagonist is an anti-PRO antibody.

In another embodiment, the invention concerns a composition of matter comprising a PRO polypeptide or an agonist or antagonist antibody which binds the polypeptide in admixture with a carrier or excipient. In one aspect, the composition comprises a therapeutically effective amount of the polypeptide or antibody. In another aspect, when the composition comprises an immune stimulating molecule, the composition is useful for: (a) increasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) stimulating or enhancing an immune response in a mammal in need thereof, (c) increasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen, (d) stimulating the activity of T-lymphocytes or (e) increasing the vascular permeability. In a further aspect, when the composition comprises an immune inhibiting molecule, the composition is useful for: (a) decreasing infiltration of inflammatory cells into a tissue of a mammal in need thereof, (b) inhibiting or reducing an

immune response in a mammal in need thereof, (c) decreasing the activity of T-lymphocytes or (d) decreasing the proliferation of T-lymphocytes in a mammal in need thereof in response to an antigen. In another aspect, the composition comprises a further active ingredient, which may, for example, be a further antibody or a cytotoxic or chemotherapeutic agent. Preferably, the composition is sterile.

5 In another embodiment, the invention concerns a method of treating an immune related disorder in a mammal in need thereof, comprising administering to the mammal an effective amount of a PRO polypeptide, an agonist thereof, or an antagonist thereto. In a preferred aspect, the immune related disorder is selected from the group consisting of: systemic lupus erythematosis, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis, idiopathic inflammatory myopathies, 10 Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia, autoimmune thrombocytopenia, thyroiditis, diabetes mellitus, immune-mediated renal disease, demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious, autoimmune chronic active hepatitis, primary biliary cirrhosis, 15 granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease, gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation 20 associated diseases including graft rejection and graft -versus-host-disease.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody. In one aspect, the present invention concerns an isolated antibody which binds a PRO polypeptide. In another aspect, the antibody mimics the activity of a 25 PRO polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a PRO polypeptide (an antagonist antibody). In another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a monoclonal antibody, a single-chain antibody, or an anti-idiotypic 30 antibody.

In yet another embodiment, the present invention provides a composition comprising an anti-PRO antibody in admixture with a pharmaceutically acceptable carrier. In one aspect, the composition comprises a therapeutically effective amount of the antibody. Preferably, the composition is sterile. The composition may be administered in the form of a liquid pharmaceutical formulation, which may be preserved to achieve 35 extended storage stability. Alternatively, the antibody is a monoclonal antibody, an antibody fragment, a humanized antibody, or a single-chain antibody.

In a further embodiment, the invention concerns an article of manufacture, comprising:

- (a) a composition of matter comprising a PRO polypeptide or agonist or antagonist thereof;
- (b) a container containing said composition; and

(c) a label affixed to said container, or a package insert included in said container referring to the use of said PRO polypeptide or agonist or antagonist thereof in the treatment of an immune related disease. The composition may comprise a therapeutically effective amount of the PRO polypeptide or the agonist or antagonist thereof.

5 In yet another embodiment, the present invention concerns a method of diagnosing an immune related disease in a mammal, comprising detecting the level of expression of a gene encoding a PRO polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a higher or lower expression level in the test sample as compared to the control sample indicates the presence of immune related disease in the mammal  
10 from which the test tissue cells were obtained.

In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-PRO antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and a PRO polypeptide, in the test sample; wherein the formation of said complex is indicative of the presence or  
15 absence of said disease. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence or absence of an immune disease in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light  
20 microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected of having a deficiency or abnormality of the immune system.

In another embodiment, the invention provides a method for determining the presence of a PRO polypeptide in a sample comprising exposing a test sample of cells suspected of containing the PRO polypeptide to an anti-PRO antibody and determining the binding of said antibody to said cell sample. In a specific aspect, the sample comprises a cell suspected of containing the PRO polypeptide and the antibody binds to the cell. The antibody is preferably detectably labeled and/or bound to a solid support.  
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In another embodiment, the present invention concerns an immune-related disease diagnostic kit, comprising an anti-PRO antibody and a carrier in suitable packaging. The kit preferably contains instructions for using the antibody to detect the presence of the PRO polypeptide. Preferably the carrier is  
30 pharmaceutically acceptable.

In another embodiment, the present invention concerns a diagnostic kit, containing an anti-PRO antibody in suitable packaging. The kit preferably contains instructions for using the antibody to detect the PRO polypeptide.

In another embodiment, the invention provides a method of diagnosing an immune-related disease in a mammal which comprises detecting the presence or absence of a PRO polypeptide in a test sample of tissue cells obtained from said mammal, wherein the presence or absence of the PRO polypeptide in said test sample is indicative of the presence of an immune-related disease in said mammal.  
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In another embodiment, the present invention concerns a method for identifying an agonist of a PRO polypeptide comprising:

(a) contacting cells and a test compound to be screened under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective agonist, wherein the induction of said cellular response is indicative of said test compound being an effective agonist.

In another embodiment, the invention concerns a method for identifying a compound capable of inhibiting the activity of a PRO polypeptide comprising contacting a candidate compound with a PRO polypeptide under conditions and for a time sufficient to allow these two components to interact and determining whether the activity of the PRO polypeptide is inhibited. In a specific aspect, either the candidate compound or the PRO polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened in the presence of a PRO polypeptide under conditions suitable for the induction of a cellular response normally induced by a PRO polypeptide; and

(b) determining the induction of said cellular response to determine if the test compound is an effective antagonist.

In another embodiment, the invention provides a method for identifying a compound that inhibits the expression of a PRO polypeptide in cells that normally express the polypeptide, wherein the method comprises contacting the cells with a test compound and determining whether the expression of the PRO polypeptide is inhibited. In a preferred aspect, this method comprises the steps of:

(a) contacting cells and a test compound to be screened under conditions suitable for allowing expression of the PRO polypeptide; and

(b) determining the inhibition of expression of said polypeptide.

In yet another embodiment, the present invention concerns a method for treating an immune-related disorder in a mammal that suffers therefrom comprising administering to the mammal a nucleic acid molecule that codes for either (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide or (c) an antagonist of a PRO polypeptide, wherein said agonist or antagonist may be an anti-PRO antibody. In a preferred embodiment, the mammal is human. In another preferred embodiment, the nucleic acid is administered via *ex vivo* gene therapy. In a further preferred embodiment, the nucleic acid is comprised within a vector, more preferably an adenoviral, adeno-associated viral, lentiviral or retroviral vector.

In yet another aspect, the invention provides a recombinant viral particle comprising a viral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide, or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein the viral vector is in association with viral structural proteins. Preferably, the signal sequence is from a mammal, such as from a native PRO polypeptide.

In a still further embodiment, the invention concerns an *ex vivo* producer cell comprising a nucleic acid construct that expresses retroviral structural proteins and also comprises a retroviral vector consisting essentially of a promoter, nucleic acid encoding (a) a PRO polypeptide, (b) an agonist polypeptide of a PRO polypeptide or (c) an antagonist polypeptide of a PRO polypeptide, and a signal sequence for cellular secretion of the polypeptide, wherein said producer cell packages the retroviral vector in association with the structural proteins to produce recombinant retroviral particles.

In a still further embodiment, the invention provides a method of increasing the activity of T-lymphocytes in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of T-lymphocytes in the mammal is increased.

5 In a still further embodiment, the invention provides a method of decreasing the activity of T-lymphocytes in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the activity of T-lymphocytes in the mammal is decreased.

10 In a still further embodiment, the invention provides a method of increasing the proliferation of T-lymphocytes in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of T-lymphocytes in the mammal is increased.

15 In a still further embodiment, the invention provides a method of decreasing the proliferation of T-lymphocytes in a mammal comprising administering to said mammal (a) a PRO polypeptide, (b) an agonist of a PRO polypeptide, or (c) an antagonist of a PRO polypeptide, wherein the proliferation of T-lymphocytes in the mammal is decreased.

**B. Additional Embodiments**

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the herein described polypeptides. Host cell comprising any such vector are also provided.

20 By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the herein described polypeptides is further provided and comprises culturing host cells under conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

25 In other embodiments, the invention provides chimeric molecules comprising any of the herein described polypeptides fused to a heterologous polypeptide or amino acid sequence. Example of such chimeric molecules comprise any of the herein described polypeptides fused to an epitope tag sequence or a Fc region of an immunoglobulin.

30 In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody, humanized antibody, antibody fragment or single-chain antibody.

In yet other embodiments, the invention provides oligonucleotide probes useful for isolating genomic and cDNA nucleotide sequences or as antisense probes, wherein those probes may be derived from any of the above or below described nucleotide sequences.

35 In other embodiments, the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a PRO polypeptide.

40 In one aspect, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity,

alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule encoding a PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In other aspects, the isolated nucleic acid molecule comprises a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule comprising the coding sequence of a full-length PRO polypeptide cDNA as disclosed herein, the coding sequence of a PRO polypeptide lacking the signal peptide as disclosed herein, the coding sequence of an extracellular domain of a transmembrane PRO polypeptide, with or without the signal peptide, as disclosed herein or the coding sequence of any other specifically defined fragment of the full-length amino acid sequence as disclosed herein, or (b) the complement of the DNA molecule of (a).

In a further aspect, the invention concerns an isolated nucleic acid molecule comprising a nucleotide sequence having at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid

sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity to (a) a DNA molecule that encodes the same mature polypeptide encoded by any of 5 the human protein cDNAs as disclosed herein, or (b) the complement of the DNA molecule of (a).

Another aspect the invention provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding a PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated, or is complementary to such encoding nucleotide sequence, wherein the transmembrane domain(s) of such polypeptide are disclosed herein. Therefore, soluble extracellular domains of the herein 10 described PRO polypeptides are contemplated.

Another embodiment is directed to fragments of a PRO polypeptide coding sequence, or the complement thereof, that may find use as, for example, hybridization probes, for encoding fragments of a PRO polypeptide that may optionally encode a polypeptide comprising a binding site for an anti-PRO antibody or as antisense oligonucleotide probes. Such nucleic acid fragments are usually at least about 20 nucleotides in length, alternatively at least about 30 nucleotides in length, alternatively at least about 40 nucleotides in length, alternatively at least about 50 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 70 nucleotides in length, alternatively at least about 80 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 100 nucleotides in length, alternatively at least about 110 nucleotides in length, alternatively at least about 120 15 nucleotides in length, alternatively at least about 130 nucleotides in length, alternatively at least about 140 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 160 nucleotides in length, alternatively at least about 170 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 190 nucleotides in length, alternatively at least about 200 nucleotides in length, alternatively at least about 250 nucleotides in length, alternatively at least about 300 20 nucleotides in length, alternatively at least about 350 nucleotides in length, alternatively at least about 400 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 500 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 700 nucleotides in length, alternatively at least about 800 nucleotides in length, alternatively at least about 900 nucleotides in length and alternatively at least about 1000 nucleotides in length, wherein in this context the 25 term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length. It is noted that novel fragments of a PRO polypeptide-encoding nucleotide sequence may be determined in a routine manner by aligning the PRO polypeptide-encoding nucleotide sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding 30 nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences herein above identified.

In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity,  
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PRO polypeptide having a full-length amino acid sequence as disclosed herein, an amino acid sequence  
15 lacking the signal peptide as disclosed herein, an extracellular domain of a transmembrane protein, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of the full-length amino acid sequence as disclosed herein.

In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity, alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity,  
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25 alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino acid sequence identity to an  
amino acid sequence encoded by any of the human protein cDNAs as disclosed herein.  
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In a specific aspect, the invention provides an isolated PRO polypeptide without the N-terminal signal sequence and/or the initiating methionine and is encoded by a nucleotide sequence that encodes such an amino acid sequence as herein before described. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

Another aspect the invention provides an isolated PRO polypeptide which is either transmembrane domain-deleted or transmembrane domain-inactivated. Processes for producing the same are also herein described, wherein those processes comprise culturing a host cell comprising a vector which comprises the  
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appropriate encoding nucleic acid molecule under conditions suitable for expression of the PRO polypeptide and recovering the PRO polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO polypeptide as defined herein. In a particular embodiment, the agonist or antagonist is an anti-PRO antibody or a small molecule.

In a further embodiment, the invention concerns a method of identifying agonists or antagonists to a PRO polypeptide which comprise contacting the PRO polypeptide with a candidate molecule and monitoring a biological activity mediated by said PRO polypeptide. Preferably, the PRO polypeptide is a native PRO polypeptide.

10 In a still further embodiment, the invention concerns a composition of matter comprising a PRO polypeptide, or an agonist or antagonist of a PRO polypeptide as herein described, or an anti-PRO antibody, in combination with a carrier. Optionally, the carrier is a pharmaceutically acceptable carrier.

Another embodiment of the present invention is directed to the use of a PRO polypeptide, or an agonist or antagonist thereof as herein before described, or an anti-PRO antibody, for the preparation of a  
15 medicament useful in the treatment of a condition which is responsive to the PRO polypeptide, an agonist or antagonist thereof or an anti-PRO antibody.

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 Figure 102A-B: DNA226870, NP\_000782.1, 48808\_at  
 Figure 103: PRO37333  
 Figure 104: DNA328366, NP\_079233.1, 59375\_at  
 Figure 105: PRO84225  
 Figure 106: DNA331437, 338326.15, 60084\_at  
 Figure 107: PRO86497  
 Figure 108: DNA328367, NP\_079108.2, 60471\_at

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Figure 110: DNA327876, NP\_005081.1, 60528\_at  
Figure 111: PRO83815  
Figure 112: DNA329917, NP\_065174.1, 64486\_at  
Figure 113: PRO85232  
Figure 114: DNA329918, BC008671, 65630\_at  
Figure 115: PRO85233  
Figure 116A-B: DNA196428, BAA31649.1, 76897\_s\_at  
Figure 117: PRO71274  
Figure 118: DNA329919, C20orf67, 89948\_at  
Figure 119: PRO85234  
Figure 120: DNA328369, BC007634, 90610\_at  
Figure 121: DNA269410, NP\_002725.1, 200605\_s\_at  
Figure 122: PRO57836  
Figure 123A-B: DNA326380, NP\_004850.1, 200614\_at  
Figure 124: PRO82774  
Figure 125A-B: DNA194778, NP\_055545.1, 200616\_s\_at  
Figure 126: PRO24056  
Figure 127: DNA287245, NP\_004175.1, 200628\_s\_at  
Figure 128: PRO69520  
Figure 129: DNA287245, WARS, 200629\_at  
Figure 130: PRO69520  
Figure 131: DNA327532, GLUL, 200648\_s\_at  
Figure 132: PRO71134  
Figure 133: DNA97285, NP\_005557.1, 200650\_s\_at  
Figure 134: PRO3632  
Figure 135: DNA226125, NP\_003136.1, 200652\_at  
Figure 136: PRO36588  
Figure 137: DNA325923, NP\_008819.1, 200655\_s\_at  
Figure 138: PRO4904  
Figure 139: DNA227055, NP\_002625.1, 200658\_s\_at  
Figure 140: PRO37518  
Figure 141: DNA275062, NP\_006136.1, 200664\_s\_at  
Figure 142: PRO62782  
Figure 143: DNA275062, DNAJB1, 200666\_s\_at  
Figure 144: PRO62782  
Figure 145A-B: DNA328372, 105551.7, 200685\_at  
Figure 146: PRO84229  
Figure 147A-B: DNA329920, NP\_036558.1, 200687\_s\_at  
Figure 148: PRO85235  
Figure 149: DNA324633, BC000478, 200691\_s\_at  
Figure 150: PRO81277  
Figure 151: DNA324897, NP\_006845.1, 200700\_s\_at  
Figure 152: PRO12468  
Figure 153: DNA275267, NP\_003737.1, 200703\_at  
Figure 154: PRO62952  
Figure 155: DNA328373, AB034747, 200704\_at  
Figure 156: PRO84230  
Figure 157: DNA328374, NP\_004853.1, 200706\_s\_at  
Figure 158: PRO84231  
Figure 159: DNA290260, NP\_036555.1, 200715\_x\_at  
Figure 160: PRO70385  
Figure 161: DNA329921, 1315403.9, 200719\_at  
Figure 162: PRO85236  
Figure 163: DNA329538, M11S1, 200722\_s\_at  
Figure 164: PRO85088  
Figure 165: DNA227618, HSGPIP137, 200723\_s\_at  
Figure 166: PRO38081  
Figure 167: DNA327114, NP\_006004.1, 200725\_x\_at  
Figure 168: PRO62466  
Figure 169A-B: DNA327534, NP\_003454.1, 200730\_s\_at  
Figure 170: PRO41180  
Figure 171A-B: DNA327534, PTP4A1, 200731\_s\_at  
Figure 172: PRO41180  
Figure 173: DNA331438, 402431.7, 200732\_s\_at  
Figure 174: PRO86498  
Figure 175: DNA327845, NP\_000282.1, 200737\_at  
Figure 176: PRO61271  
Figure 177: DNA327845, PGK1, 200738\_s\_at  
Figure 178: PRO61271  
Figure 179: DNA287207, NP\_006316.1, 200750\_s\_at  
Figure 180: PRO39268  
Figure 181A-B: DNA274977, HSU97105, 200762\_at  
Figure 182: PRO62709  
Figure 183: DNA324135, BC001854, 200768\_s\_at  
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Figure 185: DNA324135, NP\_005902.1, 200769\_s\_at  
Figure 186: PRO80837  
Figure 187: DNA271608, NP\_055485.1, 200777\_s\_at  
Figure 188: PRO59895  
Figure 189: DNA226262, NP\_005554.1, 200783\_s\_at  
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Figure 191: DNA324060, NP\_002530.1, 200790\_at  
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Figure 193: DNA272928, NP\_055579.1, 200794\_x\_at  
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Figure 195: DNA304668, NP\_005336.2, 200799\_at  
Figure 196: PRO71095  
Figure 197: DNA227607, NP\_005337.1, 200800\_s\_at  
Figure 198: PRO38070  
Figure 199: DNA287211, NP\_002147.1, 200806\_s\_at  
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Figure 201: DNA287211, HSPD1, 200807\_s\_at  
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Figure 203: DNA269874, NP\_001271.1, 200810\_s\_at  
Figure 204: PRO58272  
Figure 205: DNA269874, CIRBP, 200811\_at  
Figure 206: PRO58272  
Figure 207: DNA227795, NP\_006420.1, 200812\_at  
Figure 208: PRO38258  
Figure 209: DNA325596, NP\_000356.1, 200822\_x\_at  
Figure 210: PRO69549  
Figure 211A-B: DNA328700, AF097514, 200831\_s\_at  
Figure 212: PRO84464  
Figure 213A-B: DNA328378, AB032261, 200832\_s\_at  
Figure 214: PRO84233  
Figure 215: DNA329922, CTSB, 200838\_at  
Figure 216: PRO3344

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 Figure 218: PRO2678  
 Figure 219: DNA329923, NP\_057211.3, 200847.s.at  
 Figure 220: PRO85237  
 Figure 221: DNA324509, NP\_002097.1, 200853.at  
 Figure 222: PRO10297  
 Figure 223A-C: DNA331439, NP\_001447.1,  
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 Figure 225A-B: DNA228029, NP\_055577.1, 200862.at  
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 Figure 227: DNA226112, NP\_002769.1, 200866.s.at  
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 Figure 229: DNA226112, PSAP, 200871.s.at  
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 Figure 231: DNA254537, NP\_002957.1, 200872.at  
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 Figure 233: DNA271030, NP\_006383.1, 200875.s.at  
 Figure 234: PRO59358  
 Figure 235: DNA324107, NP\_006421.1, 200877.at  
 Figure 236: PRO80814  
 Figure 237: DNA328379, BC015869, 200878.at  
 Figure 238: PRO84234  
 Figure 239: DNA329099, 1164406.9, 200880.at  
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 Figure 242: PRO60127  
 Figure 243: DNA287187, NP\_002620.1, 200886.s.at  
 Figure 244: PRO69473  
 Figure 245A-B: DNA327539, NP\_009330.1,  
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 Figure 247: DNA326326, NP\_000969.1, 200888.s.at  
 Figure 248: PRO82724  
 Figure 249: DNA325584, NP\_002005.1, 200894.s.at  
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 Figure 251: DNA325584, FKBP4, 200895.s.at  
 Figure 252: PRO59262  
 Figure 253: DNA328380, HSHLAEHCM, 200904.at  
 Figure 254: DNA304665, NP\_000995.1, 200909.s.at  
 Figure 255: PRO71092  
 Figure 256: DNA272695, NP\_001722.1, 200920.s.at  
 Figure 257: PRO60817  
 Figure 258: DNA272695, BTG1, 200921.s.at  
 Figure 259: PRO60817  
 Figure 260: DNA227077, NP\_005558.1, 200923.at  
 Figure 261: PRO37540  
 Figure 262: DNA327255, NP\_002385.2, 200924.s.at  
 Figure 263: PRO57298  
 Figure 264: DNA225878, NP\_004334.1, 200935.at  
 Figure 265: PRO36341  
 Figure 266: DNA329925, NP\_001528.1, 200942.s.at  
 Figure 267: PRO85239  
 Figure 268A-B: DNA287217, NP\_001750.1,  
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 Figure 269: PRO36766
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 Figure 271: PRO36766  
 Figure 272: DNA227491, NP\_009027.1, 200960.x.at  
 Figure 273: PRO37954  
 Figure 274: DNA331440, NP\_036380.2, 200961.at  
 Figure 275: PRO86500  
 Figure 276A-B: DNA331289, ABLIM1, 200965.s.at  
 Figure 277: PRO86390  
 Figure 278: DNA287355, NP\_000025.1, 200966.x.at  
 Figure 279: PRO69617  
 Figure 280: DNA324110, NP\_005908.1, 200978.at  
 Figure 281: PRO4918  
 Figure 282: DNA329928, ANXA6, 200982.s.at  
 Figure 283: PRO85241  
 Figure 284A-B: DNA325896, NP\_001521.1, 200989.at  
 Figure 285: PRO82352  
 Figure 286: DNA329929, 400903.6, 200994.at  
 Figure 287: PRO85242  
 Figure 288: DNA325778, CKAP4, 200998.s.at  
 Figure 289: PRO82248  
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 Figure 291: DNA275408, NP\_001596.1, 201000.at  
 Figure 292: PRO63068  
 Figure 293: DNA304713, NP\_006463.2, 201008.s.at  
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 Figure 295: DNA304713, TXNIP, 201009.s.at  
 Figure 296: PRO71139  
 Figure 297: DNA304713, S73591, 201010.s.at  
 Figure 298: PRO71139  
 Figure 299: DNA89242, NP\_000691.1, 201012.at  
 Figure 300: PRO2907  
 Figure 301: DNA328388, BC010273, 201013.s.at  
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 Figure 303: DNA328388, NP\_006443.1, 201014.s.at  
 Figure 304: PRO84240  
 Figure 305: DNA151697, DNA151697, 201016.at  
 Figure 306: PRO11993  
 Figure 307A-B: DNA329101, NP\_056988.2,  
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 Figure 308: PRO84751  
 Figure 309A-B: DNA329101, IF2, 201027.s.at  
 Figure 310: PRO84751  
 Figure 311: DNA287372, NP\_002618.1, 201037.at  
 Figure 312: PRO69632  
 Figure 313: DNA328391, NP\_004408.1, 201041.s.at  
 Figure 314: PRO84242  
 Figure 315: DNA328391, DUSP1, 201044.x.at  
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 Figure 317: DNA274743, NP\_002850.1, 201087.at  
 Figure 318: PRO62517  
 Figure 319: DNA254725, NP\_002257.1, 201088.at  
 Figure 320: PRO49824  
 Figure 321: DNA329930, ATP6V1B2, 201089.at  
 Figure 322: PRO85243  
 Figure 323: DNA287198, NP\_006073.1, 201090.x.at  
 Figure 324: PRO69484

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Figure 326: PRO84245  
Figure 327: DNA304719, NP\_002296.1, 201105\_at  
Figure 328: PRO71145  
Figure 329: DNA329931, AF053642, 201111\_at  
Figure 330: DNA331442, NP\_002783.1, 201114\_x\_at  
Figure 331: PRO83189  
Figure 332: DNA273865, NP\_006221.1, 201115\_at  
Figure 333: PRO61824  
Figure 334: DNA326273, NP\_001961.1, 201123\_s\_at  
Figure 335: PRO82678  
Figure 336: DNA329255, NP\_006267.1, 201129\_at  
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Figure 338: DNA329103, NP\_002112.2, 201137\_s\_at  
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Figure 340: DNA329104, NP\_004085.1, 201144\_s\_at  
Figure 341: PRO69550  
Figure 342: DNA329105, NP\_006109.2, 201145\_at  
Figure 343: PRO84753  
Figure 344: DNA329015, NFE2L2, 201146\_at  
Figure 345: PRO84691  
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Figure 347: PRO85244  
Figure 348: DNA151802, NP\_003661.1, 201169\_s\_at  
Figure 349: PRO12890  
Figure 350: DNA151802, BHLHB2, 201170\_s\_at  
Figure 351: PRO12890  
Figure 352: DNA273342, NP\_005887.1, 201193\_at  
Figure 353: PRO61345  
Figure 354: DNA331443, NP\_003000.1, 201194\_at  
Figure 355: PRO86501  
Figure 356A-B: DNA103453, HUME16GEN, 201195\_s\_at  
Figure 357: PRO4780  
Figure 358: DNA272251, NP\_002798.1, 201198\_s\_at  
Figure 359: PRO60513  
Figure 360: DNA103488, NP\_002583.1, 201202\_at  
Figure 361: PRO4815  
Figure 362: DNA327544, NP\_002865.1, 201222\_s\_at  
Figure 363: PRO70357  
Figure 364: DNA287173, ENO1, 201231\_s\_at  
Figure 365: PRO69463  
Figure 366: DNA287331, NP\_002645.1, 201251\_at  
Figure 367: PRO69595  
Figure 368: DNA274139, NP\_006494.1, 201252\_at  
Figure 369: PRO62075  
Figure 370: DNA270950, NP\_003182.1, 201263\_at  
Figure 371: PRO59281  
Figure 372A-B: DNA328404, NP\_003321.1, 201266\_at  
Figure 373: PRO84251  
Figure 374: DNA331444, NP\_002781.1, 201274\_at  
Figure 375: PRO60981  
Figure 376: DNA323936, NP\_001995.1, 201275\_at  
Figure 377: PRO80669  
Figure 378: DNA328405, NP\_112556.1, 201277\_s\_at  
Figure 379: PRO84252  
Figure 380: DNA270526, NP\_001166.1, 201288\_at  
Figure 381: PRO58903  
Figure 382A-B: DNA327545, TOP2A, 201291\_s\_at  
Figure 383: PRO82731  
Figure 384: DNA327546, HSTOP2A10, 201292\_at  
Figure 385: DNA328407, WSB1, 201294\_s\_at  
Figure 386: PRO84254  
Figure 387A-B: DNA226778, HSM800772, 201295\_s\_at  
Figure 388: PRO37241  
Figure 389: DNA327547, NP\_060691.1, 201298\_s\_at  
Figure 390: PRO83583  
Figure 391: DNA287222, NP\_055555.1, 201303\_at  
Figure 392: PRO69501  
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Figure 394: PRO81261  
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Figure 396: PRO38010  
Figure 397: DNA331445, NP\_002778.1, 201317\_s\_at  
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Figure 399: DNA274745, NP\_006815.1, 201323\_at  
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Figure 403: DNA329002, AF385084, 201326\_at  
Figure 404: PRO4912  
Figure 405: DNA329002, NP\_001753.1, 201327\_s\_at  
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Figure 407: DNA269536, S80343, 201330\_at  
Figure 408: PRO57952  
Figure 409: DNA273323, NP\_004243.1, 201349\_at  
Figure 410: PRO61330  
Figure 411: DNA103227, NP\_004466.1, 201350\_at  
Figure 412: PRO4557  
Figure 413: DNA329934, NP\_000090.1, 201360\_at  
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Figure 415: DNA329107, NP\_008818.3, 201367\_s\_at  
Figure 416: PRO84754  
Figure 417A-B: DNA329108, 1383643.16, 201368\_at  
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Figure 419: DNA329107, ZFP36L2, 201369\_s\_at  
Figure 420: PRO84754  
Figure 421A-E: DNA331446, NP\_000436.1, 201373\_at  
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Figure 423: DNA329109, NP\_004957.1, 201376\_s\_at  
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Figure 425: DNA329111, NP\_001349.1, 201385\_at  
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Figure 432: PRO61014  
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Figure 436: PRO70544  
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Figure 438: PRO85248  
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Figure 440: PRO2592  
Figure 441: DNA226600, NP\_003371.1, 201426\_s\_at  
Figure 442: PRO37063  
Figure 443: DNA272286, NP\_001743.1, 201432\_at  
Figure 444: PRO60544  
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Figure 447A-C: DNA88140, COL6A3, 201438\_at  
Figure 448: PRO2670  
Figure 449: DNA150535, NP\_004809.1, 201440\_at  
Figure 450: PRO12078  
Figure 451: DNA325049, NP\_005605.1, 201453\_x\_at  
Figure 452: PRO37938  
Figure 453: DNA326736, NP\_006657.1, 201459\_at  
Figure 454: PRO83076  
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Figure 456: PRO36822  
Figure 457: DNA226359, JUN, 201465\_s\_at  
Figure 458: PRO36822  
Figure 459: DNA226359, DNA226359, 201466\_s\_at  
Figure 460: PRO36822  
Figure 461: DNA328413, NP\_004823.1, 201470\_at  
Figure 462: PRO84258  
Figure 463: DNA103320, NP\_002220.1, 201473\_at  
Figure 464: PRO4650  
Figure 465: DNA325704, NP\_004981.2, 201475\_x\_at  
Figure 466: PRO82188  
Figure 467: DNA327551, NP\_001024.1, 201476\_s\_at  
Figure 468: PRO59289  
Figure 469: DNA327551, RRM1, 201477\_s\_at  
Figure 470: PRO59289  
Figure 471: DNA254783, NP\_001354.1, 201478\_s\_at  
Figure 472: PRO49881  
Figure 473: DNA329940, NP\_001805.1, 201487\_at  
Figure 474: PRO2679  
Figure 475: DNA304459, BC005020, 201489\_at  
Figure 476: PRO37073  
Figure 477: DNA304459, NP\_005720.1, 201490\_s\_at  
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Figure 480: PRO82373  
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Figure 482: PRO60207  
Figure 483: DNA329941, NP\_001543.1, 201508\_at  
Figure 484: PRO85249  
Figure 485: DNA323741, NP\_003123.1, 201516\_at  
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Figure 489: DNA328418, NP\_003398.1, 201531\_at  
Figure 490: PRO84261  
Figure 491: DNA331292, NP\_002779.1, 201532\_at  
Figure 492: PRO84262  
Figure 493: DNA329943, NP\_009037.1, 201534\_s\_at  
Figure 494: PRO85251  
Figure 495: DNA331448, UBL3, 201535\_at  
Figure 496: PRO86503  
Figure 497: DNA272171, NP\_002379.2, 201555\_at  
Figure 498: PRO60438  
Figure 499: DNA226291, NP\_055047.1, 201556\_s\_at  
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Figure 502: PRO36754  
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Figure 504: PRO59324  
Figure 505: DNA227071, NP\_000260.1, 201577\_at  
Figure 506: PRO37534  
Figure 507: DNA327199, NP\_066979.1, 201580\_s\_at  
Figure 508: PRO83475  
Figure 509A-B: DNA329944, AB032988, 201581\_at  
Figure 510: DNA329945, NP\_006354.2, 201583\_s\_at  
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Figure 513: DNA290280, NP\_004359.1, 201605\_x\_at  
Figure 514: PRO70425  
Figure 515: DNA329947, NP\_536806.1, 201613\_s\_at  
Figure 516: PRO37674  
Figure 517: DNA272904, NP\_006784.1, 201619\_at  
Figure 518: PRO60991  
Figure 519: DNA255406, NP\_005533.1, 201625\_s\_at  
Figure 520: PRO50473  
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Figure 522: PRO50473  
Figure 523: DNA329115, NP\_434702.1, 201631\_s\_at  
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Figure 534: PRO61695  
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 Figure 545: DNA324742, NP\_001751.1, 201700\_s\_at  
 Figure 546: PRO81367  
 Figure 547: DNA270883, NP\_001061.1, 201714\_s\_at  
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 Figure 551: DNA227461, NP\_006753.1, 201720\_s\_at  
 Figure 552: PRO37924  
 Figure 553: DNA227461, LAPTMS, 201721\_s\_at  
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 Figure 555: DNA329949, BC003376, 201726\_s\_at  
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 Figure 575: DNA329950, NP\_076961.1, 201764\_s\_at  
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 Figure 585: DNA324015, NP\_006326.1, 201821\_s\_at  
 Figure 586: PRO80735  
 Figure 587: DNA329952, BC010285, 201829\_s\_at  
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 Figure 591: DNA329954, NP\_001518.1, 201833\_s\_at  
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 Figure 595: DNA254350, NP\_004043.2, 201848\_s\_at  
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 Figure 597: DNA254350, BNIP3, 201849\_s\_at  
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 Figure 599: DNA329118, NP\_068660.1, 201853\_s\_at  
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 Figure 601: DNA272066, NP\_002931.1, 201872\_s\_at  
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 Figure 609: DNA328431, NP\_001817.1, 201897\_s\_at  
 Figure 610: PRO45093  
 Figure 611: DNA254978, NP\_060625.1, 201917\_s\_at  
 Figure 612: PRO50067  
 Figure 613: DNA329057, NP\_004116.2, 201921\_s\_at  
 Figure 614: PRO84719  
 Figure 615: DNA227112, NP\_006397.1, 201923\_s\_at  
 Figure 616: PRO37575  
 Figure 617: DNA275240, NP\_005906.2, 201930\_s\_at  
 Figure 618: PRO62927  
 Figure 619: DNA273014, NP\_000117.1, 201931\_s\_at  
 Figure 620: PRO61085  
 Figure 621A-B: DNA329120, NP\_002560.1, 201945\_s\_at  
 Figure 622: PRO2752  
 Figure 623: DNA274167, NP\_006422.1, 201946\_s\_at  
 Figure 624: PRO62097  
 Figure 625: DNA274167, CCT2, 201947\_s\_at  
 Figure 626: PRO62097  
 Figure 627: DNA103481, NP\_037417.1, 201948\_s\_at  
 Figure 628: PRO4808  
 Figure 629A-B: DNA327563, NP\_066945.1, 201963\_s\_at  
 Figure 630: PRO83592  
 Figure 631: DNA275214, NP\_002473.1, 201970\_s\_at  
 Figure 632: PRO62908  
 Figure 633A-B: DNA328433, ATP6V1A1, 201971\_s\_at  
 Figure 634: PRO84268  
 Figure 635A-B: DNA272191, NP\_002947.1, 201975\_s\_at  
 Figure 636: PRO60456  
 Figure 637: DNA328809, PTPN12, 202006\_s\_at  
 Figure 638: PRO4803  
 Figure 639: DNA328437, AF083441, 202021\_x\_at  
 Figure 640: PRO84271  
 Figure 641: DNA329957, NP\_005156.1, 202022\_s\_at  
 Figure 642: PRO85261  
 Figure 643A-B: DNA329958, NP\_510880.1, 202039\_s\_at  
 Figure 644: PRO85262  
 Figure 645: DNA327017, NP\_004586.2, 202043\_s\_at  
 Figure 646: PRO61744  
 Figure 647A-B: DNA227985, NP\_055107.1, 202047\_s\_at  
 Figure 648: PRO38448  
 Figure 649A-B: DNA225991, NP\_000518.1, 202067\_s\_at  
 Figure 650: PRO36454

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Figure 653: DNA327567, NP\_005521.1, 202069.s.at  
Figure 654: PRO83596  
Figure 655: DNA226116, NP\_002990.1, 202071.at  
Figure 656: PRO36579  
Figure 657: DNA289522, NP\_004994.1, 202077.at  
Figure 658: PRO70276  
Figure 659: DNA327568, NP\_002453.1, 202086.at  
Figure 660: PRO57922  
Figure 661: DNA327569, CTSL, 202087.s.at  
Figure 662: PRO2683  
Figure 663: DNA329959, 251651.5, 202094.at  
Figure 664: PRO85263  
Figure 665: DNA129504, NP\_001159.1, 202095.s.at  
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Figure 667: DNA328440, NP\_004517.1, 202107.s.at  
Figure 668: PRO84274  
Figure 669: DNA329960, 1381890.1, 202136.at  
Figure 670: PRO85264  
Figure 671: DNA324895, NP\_006294.2, 202138.x.at  
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Figure 673: DNA227150, NP\_002337.1, 202145.at  
Figure 674: PRO37613  
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Figure 676: PRO84695  
Figure 677: DNA328442, NP\_006078.2, 202154.x.at  
Figure 678: PRO84275  
Figure 679A-C: DNA331449, NP\_004371.1, 202160.at  
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Figure 695: DNA329965, BC001051, 202208.s.at  
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Figure 699: DNA328258, SLC16A1, 202236.s.at  
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Figure 703: DNA328444, MGC14458, 202246.s.at  
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Figure 707: DNA326120, NP\_006101.1, 202257.s.at  
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Figure 709: DNA150808, NP\_002044.1, 202269.x.at  
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Figure 711: DNA150808, GBP1, 202270.at  
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Figure 713: DNA329966, NP\_006295.1, 202276.at  
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Figure 715: DNA304716, NP\_510867.1, 202284.s.at  
Figure 716: PRO71142  
Figure 717: DNA331450, NP\_004381.1, 202295.s.at  
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Figure 720: PRO85270  
Figure 721: DNA329524, NP\_000584.2, 202307.s.at  
Figure 722: PRO36996  
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Figure 724: PRO12105  
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Figure 731: DNA331451, UNG, 202330.s.at  
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Figure 738: PRO81689  
Figure 739: DNA270502, NP\_002807.1, 202352.s.at  
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Figure 748: PRO69635  
Figure 749: DNA150989, NP\_005523.1, 202411.at  
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 Figure 801: DNA329977, BC001553, 202536\_at  
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 Figure 816: DNA325587, NP\_068772.1, 202580\_x\_at  
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 Figure 1081: DNA28759, NP\_006150.1, 203413\_at  
 Figure 1082: PRO2520  
 Figure 1083: DNA287267, NP\_001228.1, 203418\_at  
 Figure 1084: PRO37015  
 Figure 1085A-B: DNA256807, NP\_057339.1, 203420\_at  
 Figure 1086: PRO51738  
 Figure 1087: DNA326745, NP\_002682.1, 203422\_at  
 Figure 1088: PRO83083  
 Figure 1089: DNA330009, NP\_054753.1, 203428\_s.at  
 Figure 1090: PRO85297  
 Figure 1091A-B: DNA275186, DNA275186, 203432\_at  
 Figure 1092A-B: DNA330010, NP\_005721.2, 203445\_s.at  
 Figure 1093: PRO85298  
 Figure 1094: DNA273410, NP\_004036.1, 203454\_s.at  
 Figure 1095: PRO61409  
 Figure 1096: DNA328495, NP\_055578.1, 203465\_at  
 Figure 1097: PRO58967  
 Figure 1098A-C: DNA331461, NP\_005493.2, 203504\_s.at  
 Figure 1099: PRO86511  
 Figure 1100A-B: DNA331462, NP\_003096.1, 203509\_at  
 Figure 1101: PRO86512  
 Figure 1102: DNA272911, NP\_006545.1, 203517\_at  
 Figure 1103: PRO60997  
 Figure 1104A-D: DNA331463, NP\_000072.1, 203518\_at  
 Figure 1105: PRO86513  
 Figure 1106A-C: DNA331464, NP\_055160.1, 203520\_s.at  
 Figure 1107: PRO86514  
 Figure 1108A-C: DNA330014, HRIHFB2436, 203521\_s.at  
 Figure 1109: PRO85302  
 Figure 1110: DNA325404, NP\_002330.1, 203523\_at  
 Figure 1111: PRO81936  
 Figure 1112: DNA323910, NP\_002956.1, 203535\_at  
 Figure 1113: PRO80648  
 Figure 1114A-B: DNA272399, NP\_001197.1, 203542\_s.at  
 Figure 1115: PRO60653  
 Figure 1116A-B: DNA272399, BTEB1, 203543\_s.at  
 Figure 1117: PRO60653  
 Figure 1118: DNA324684, NP\_004210.1, 203554\_x.at  
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 Figure 1120: DNA330015, NP\_004620.1, 203564\_at  
 Figure 1121: PRO58704  
 Figure 1122: DNA330016, NP\_006346.1, 203567\_s.at  
 Figure 1123: PRO85303  
 Figure 1124A-B: DNA150765, NP\_003974.1, 203579\_s.at  
 Figure 1125: PRO12458  
 Figure 1126: DNA273676, NP\_055488.1, 203584\_at  
 Figure 1127: PRO61644  
 Figure 1128: DNA271003, NP\_003720.1, 203594\_at  
 Figure 1129: PRO59332  
 Figure 1130A-B: DNA270323, NP\_036552.1, 203595\_s.at  
 Figure 1131: PRO58710  
 Figure 1132A-B: DNA270323, RI58, 203596\_s.at  
 Figure 1133: PRO58710  
 Figure 1134: DNA330017, NP\_009118.1, 203597\_s.at  
 Figure 1135: PRO60916  
 Figure 1136: DNA329604, NP\_003127.1, 203605\_at  
 Figure 1137: PRO85134  
 Figure 1138: DNA287246, NP\_004044.2, 203612\_at  
 Figure 1139: PRO69521  
 Figure 1140: DNA330018, NP\_064528.1, 203622\_s.at  
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 Figure 1142: DNA331465, SKP2, 203625\_x.at  
 Figure 1143: PRO81225  
 Figure 1144A-B: DNA327596, 345314.2, 203628\_at  
 Figure 1145: PRO1920  
 Figure 1146A-B: DNA331466, BCL2, 203685\_at  
 Figure 1147: PRO86515  
 Figure 1148A-B: DNA330021, NP\_001940.1, 203693\_s.at  
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 Figure 1154: DNA329144, KIAA0020, 203712\_at  
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 Figure 1156: DNA326402, NP\_004515.1, 203713\_s.at  
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 Figure 1158: DNA324183, DPP4, 203716\_s.at  
 Figure 1159: PRO80881  
 Figure 1160: DNA150784, NP\_001974.1, 203720\_s.at  
 Figure 1161: PRO12800  
 Figure 1162A-B: DNA269573, NP\_002212.1, 203723\_at  
 Figure 1163: PRO57986  
 Figure 1164: DNA330023, NP\_001915.1, 203725\_at  
 Figure 1165: PRO85308

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Figure 1169: PRO81905  
Figure 1170A-B: DNA150748, NP\_001105.1, 203741\_s\_at  
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Figure 1173: PRO38028  
Figure 1174: DNA330024, NP\_058521.1, 203748\_x\_at  
Figure 1175: PRO85309  
Figure 1176: DNA97279, NP\_005345.2, 203751\_x\_at  
Figure 1177: PRO3628  
Figure 1178A-B: DNA325972, BUB1B, 203755\_at  
Figure 1179: PRO82417  
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Figure 1182: DNA330026, NP\_005899.1, 203778\_at  
Figure 1183: PRO85311  
Figure 1184: DNA330027, NP\_036578.1, 203787\_at  
Figure 1185: PRO85312  
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Figure 1188: DNA331468, DGUOK, 203816\_at  
Figure 1189: PRO86517  
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Figure 1191: PRO62061  
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Figure 1193: PRO86518  
Figure 1194A-B: DNA325529, GAB2, 203853\_s\_at  
Figure 1195: PRO82037  
Figure 1196A-B: DNA275079, NP\_056648.1, 203865\_s\_at  
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Figure 1205: PRO37815  
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Figure 1210: DNA271676, NP\_002052.1, 203925\_at  
Figure 1211: PRO59961  
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Figure 1213: PRO69507  
Figure 1214: DNA330031, NP\_057210.1, 203960\_s\_at  
Figure 1215: PRO85316  
Figure 1216: DNA275012, NP\_004679.1, 203964\_at  
Figure 1217: PRO62740  
Figure 1218: DNA272338, NP\_001245.1, 203967\_at  
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Figure 1220: DNA272338, CDC6, 203968\_s\_at  
Figure 1221: PRO60595  
Figure 1222: DNA227232, NP\_001850.1, 203971\_at  
Figure 1223: PRO37695  
Figure 1224: DNA271374, NP\_005474.1, 203976\_s\_at  
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Figure 1227: PRO36596  
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Figure 1230: DNA330032, HUMGCRFC, 204007\_at  
Figure 1231: PRO85317  
Figure 1232: DNA329145, DUSP4, 204014\_at  
Figure 1233: PRO84780  
Figure 1234: DNA331470, HSU48807, 204015\_s\_at  
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Figure 1238: DNA330033, NP\_056492.1, 204019\_s\_at  
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Figure 1245: PRO85320  
Figure 1246: DNA325181, CLTA, 204050\_s\_at  
Figure 1247: PRO81742  
Figure 1248: DNA226342, NP\_000305.1, 204054\_at  
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Figure 1250A-B: DNA331471, NP\_055498.1, 204063\_s\_at  
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Figure 1252: DNA274783, NP\_006272.1, 204068\_at  
Figure 1253: PRO62549  
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Figure 1258: DNA216689, NP\_002975.1, 204103\_at  
Figure 1259: PRO34276  
Figure 1260: DNA328522, NP\_001769.2, 204118\_at  
Figure 1261: PRO2696  
Figure 1262: DNA150529, NP\_003323.1, 204122\_at  
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Figure 1264: DNA328524, NP\_057097.1, 204125\_at  
Figure 1265: PRO84336  
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Figure 1267: PRO71058  
Figure 1268: DNA330037, BC000149, 204127\_at  
Figure 1269: PRO82290

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 Figure 1272: DNA328525, BC021224, 204131\_s\_at  
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 Figure 1276: DNA330038, BC016330, 204146\_at  
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 Figure 1279: PRO85323  
 Figure 1280: DNA330039, MFNG, 204153\_s\_at  
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 Figure 1285: PRO61661  
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 Figure 1290: DNA272655, NP\_001818.1, 204170\_s\_at  
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 Figure 1292: DNA330041, NP\_000088.2, 204172\_at  
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 Figure 1294: DNA328528, MLC1SA, 204173\_at  
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 Figure 1296: DNA329148, NP\_056955.1, 204175\_at  
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 Figure 1312: DNA270434, NP\_006434.1, 204238\_s\_at  
 Figure 1313: PRO58814  
 Figure 1314A-B: DNA287273, NP\_006435.1, 204240\_s\_at  
 Figure 1315: PRO69545  
 Figure 1316: DNA330043, NP\_001789.2, 204252\_at  
 Figure 1317: PRO85326  
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 Figure 1319: PRO4854  
 Figure 1320A-B: DNA103527, VDR, 204254\_s\_at  
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 Figure 1324: DNA228132, NP\_076995.1, 204256\_at  
 Figure 1325: PRO38595  
 Figure 1326: DNA226577, NP\_071390.1, 204265\_s\_at  
 Figure 1327: PRO37040  
 Figure 1328: DNA88643, SGSH, 204293\_at  
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 Figure 1332: DNA330045, NP\_005943.1, 204326\_x\_at  
 Figure 1333: PRO82583  
 Figure 1334: DNA328530, NP\_009198.2, 204328\_at  
 Figure 1335: PRO24118  
 Figure 1336: DNA330046, 987987.10, 204334\_at  
 Figure 1337: PRO85328  
 Figure 1338: DNA328531, NP\_037542.1, 204348\_s\_at  
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 Figure 1344: DNA328533, NP\_003647.1, 204392\_at  
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 Figure 1346: DNA226462, NP\_002241.1, 204401\_at  
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 Figure 1348A-B: DNA330048, AF080255, 204407\_at  
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 Figure 1356: DNA327617, NP\_006811.1, 204439\_at  
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 Figure 1374A-B: DNA331474, 357276.11, 204552\_at

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 Figure 1418A-B: DNA256461, NP\_009017.1, 204728\_s\_at  
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 Figure 1420A-C: DNA274487, NP\_055562.1, 204730\_at  
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 Figure 1426: DNA330057, NP\_005941.1, 204745\_x\_at  
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Figure 1550: DNA299899, NP\_002148.1, 205133\_s\_at  
Figure 1551: PRO62760  
Figure 1552: DNA331482, NP\_001241.1, 205153\_s\_at  
Figure 1553: PRO34457  
Figure 1554: DNA330075, CDC25C, 205167\_s\_at  
Figure 1555: PRO85354  
Figure 1556: DNA330076, NP\_005410.1, 205170\_at  
Figure 1557: PRO85355  
Figure 1558: DNA328810, NP\_001770.1, 205173\_x\_at  
Figure 1559: PRO2557  
Figure 1560: DNA330077, ITGB3BP, 205176\_s\_at  
Figure 1561: PRO85356  
Figure 1562: DNA151804, NP\_006500.1, 205205\_at  
Figure 1563: PRO12188  
Figure 1564: DNA272443, NP\_055531.1, 205213\_at  
Figure 1565: PRO60693  
Figure 1566: DNA273535, NP\_004217.1, 205214\_at  
Figure 1567: PRO61515  
Figure 1568: DNA325255, NP\_001994.2, 205237\_at  
Figure 1569: PRO1910  
Figure 1570: DNA330078, NP\_001648.1, 205239\_at  
Figure 1571: PRO46  
Figure 1572: DNA327634, NP\_005129.1, 205241\_at  
Figure 1573: PRO83636  
Figure 1574: DNA188333, NP\_006410.1, 205242\_at  
Figure 1575: PRO21708  
Figure 1576: DNA227081, NP\_000390.2, 205249\_at  
Figure 1577: PRO37544  
Figure 1578: DNA227447, NP\_003193.1, 205254\_x\_at  
Figure 1579: PRO37910  
Figure 1580: DNA227447, TCF7, 205255\_x\_at  
Figure 1581: PRO37910  
Figure 1582A-B: DNA226483, NP\_000892.1,  
205259\_at  
Figure 1583: PRO36946

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 Figure 1585: PRO1162  
 Figure 1586A-B: DNA188301, NP\_002300.1, 205266\_at  
 Figure 1587: PRO21834  
 Figure 1588: DNA227173, NP\_001456.1, 205285\_s\_at  
 Figure 1589: PRO37636  
 Figure 1590A-B: DNA331483, CDC14A, 205288\_at  
 Figure 1591: PRO86528  
 Figure 1592A-B: DNA331484, NP\_000869.1, 205291\_at  
 Figure 1593: PRO3276  
 Figure 1594: DNA88119, NP\_000617.1, 205297\_s\_at  
 Figure 1595: PRO2663  
 Figure 1596A-B: DNA330081, NP\_003026.1, 205339\_at  
 Figure 1597: PRO85358  
 Figure 1598: DNA256854, NP\_000456.1, 205345\_at  
 Figure 1599: PRO51785  
 Figure 1600: DNA270415, NP\_002059.1, 205349\_at  
 Figure 1601: PRO58796  
 Figure 1602: DNA325568, NP\_001265.1, 205393\_s\_at  
 Figure 1603: PRO12187  
 Figure 1604: DNA325568, CHEK1, 205394\_at  
 Figure 1605: PRO12187  
 Figure 1606: DNA330082, NP\_005582.1, 205395\_s\_at  
 Figure 1607: PRO60497  
 Figure 1608: DNA328561, NP\_004624.1, 205403\_at  
 Figure 1609: PRO2019  
 Figure 1610: DNA329010, NP\_004942.1, 205419\_at  
 Figure 1611: PRO23370  
 Figure 1612A-B: DNA210654, NP\_055726.1, 205434\_s\_at  
 Figure 1613: PRO54603  
 Figure 1614: DNA287337, NP\_002096.1, 205436\_s\_at  
 Figure 1615: PRO69600  
 Figure 1616: DNA330083, NP\_003073.1, 205443\_at  
 Figure 1617: PRO69499  
 Figure 1618: DNA272221, NP\_037431.1, 205449\_at  
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 Figure 1620: DNA88194, NP\_000724.1, 205456\_at  
 Figure 1621: PRO2220  
 Figure 1622: DNA188355, NP\_004582.1, 205476\_at  
 Figure 1623: PRO21885  
 Figure 1624: DNA287224, NP\_005092.1, 205483\_s\_at  
 Figure 1625: PRO69503  
 Figure 1626: DNA330084, NP\_055265.1, 205484\_at  
 Figure 1627: PRO9895  
 Figure 1628: DNA225959, NP\_006135.1, 205488\_at  
 Figure 1629: PRO36422  
 Figure 1630: DNA331485, GNLY, 205495\_s\_at  
 Figure 1631: PRO86529  
 Figure 1632: DNA328566, NP\_060446.1, 205510\_s\_at  
 Figure 1633: PRO84363  
 Figure 1634: DNA327639, NP\_001053.2, 205513\_at  
 Figure 1635: PRO83640  
 Figure 1636: DNA330085, D86324, 205518\_s\_at  
 Figure 1637: PRO85359  
 Figure 1638: DNA330086, NP\_079184.1, 205519\_at  
 Figure 1639: PRO85360  
 Figure 1640: DNA254810, NP\_056536.1, 205527\_s\_at  
 Figure 1641: PRO49906  
 Figure 1642: DNA331486, OAS1, 205552\_s\_at  
 Figure 1643: PRO69559  
 Figure 1644: DNA330087, PCSK5, 205559\_s\_at  
 Figure 1645: PRO85361  
 Figure 1646: DNA256257, NP\_055213.1, 205569\_at  
 Figure 1647: PRO51301  
 Figure 1648A-B: DNA327643, NP\_055712.1, 205594\_at  
 Figure 1649: PRO83644  
 Figure 1650: DNA329013, NP\_005649.1, 205599\_at  
 Figure 1651: PRO20128  
 Figure 1652: DNA324324, NP\_000679.1, 205633\_s\_at  
 Figure 1653: PRO81000  
 Figure 1654: DNA330088, NP\_003087.1, 205644\_s\_at  
 Figure 1655: PRO61962  
 Figure 1656: DNA287317, NP\_003724.1, 205660\_at  
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 Figure 1672: DNA331318, SLC27A2, 205769\_at  
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 Figure 1682: DNA76517, NP\_002176.1, 205798\_at  
 Figure 1683: PRO2541  
 Figure 1684A-B: DNA271915, NP\_056191.1, 205801\_s\_at  
 Figure 1685: PRO60192  
 Figure 1686: DNA194766, NP\_079504.1, 205804\_s\_at  
 Figure 1687: PRO24046

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 Figure 1690A-B: DNA328574, JAK2, 205842\_s\_at  
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 Figure 1692: DNA330094, TREX1, 205875\_s\_at  
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 Figure 1694: DNA331320, HSU37122, 205882\_x\_at  
 Figure 1695: PRO86409  
 Figure 1696A-B: DNA220746, NP\_000876.1, 205884\_at  
 Figure 1697: PRO34724  
 Figure 1698A-B: DNA220746, ITGA4, 205885\_s\_at  
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 Figure 1702: DNA330095, NP\_004732.1, 205895\_s\_at  
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 Figure 1704: DNA328576, HSU20350, 205898\_at  
 Figure 1705: PRO4940  
 Figure 1706: DNA287318, NP\_002683.1, 205909\_at  
 Figure 1707: PRO69583  
 Figure 1708: DNA75525, NP\_005805.1, 205929\_at  
 Figure 1709: PRO2524  
 Figure 1710: DNA76516, NP\_000556.1, 205945\_at  
 Figure 1711: PRO2022  
 Figure 1712: DNA329047, NP\_006390.1, 205965\_at  
 Figure 1713: PRO58425  
 Figure 1714: DNA273487, NP\_004785.1, 206039\_at  
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 Figure 1716A-B: DNA290265, NP\_003421.1, 206059\_at  
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 Figure 1718: DNA330096, NP\_057051.1, 206060\_s\_at  
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 Figure 1722: DNA270851, NP\_006617.1, 206098\_at  
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 Figure 1724: DNA226105, NP\_002925.1, 206111\_at  
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 Figure 1726: DNA83063, NP\_004429.1, 206114\_at  
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 Figure 1734: DNA331487, GABPB2, 206173\_x\_at  
 Figure 1735: PRO86530  
 Figure 1736: DNA329005, NP\_003028.1, 206181\_at  
 Figure 1737: PRO12612  
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 Figure 1740: DNA329168, CLC, 206207\_at  
 Figure 1741: PRO84794  
 Figure 1742: DNA281446, NP\_031394.1, 206220\_s\_at  
 Figure 1743: PRO66285  
 Figure 1744: DNA281446, GAP1IP4BP, 206221\_at  
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 Figure 1748: DNA327661, NP\_005522.1, 206332\_s\_at  
 Figure 1749: PRO83652  
 Figure 1750: DNA218278, NP\_000408.1, 206341\_at  
 Figure 1751: PRO34330  
 Figure 1752: DNA269870, NP\_005382.1, 206348\_s\_at  
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 Figure 1754A-B: DNA330100, NP\_055690.1, 206364\_at  
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 Figure 1756: DNA329169, NP\_002986.1, 206366\_x\_at  
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 Figure 1758: DNA271310, NP\_004411.1, 206374\_at  
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 Figure 1760A-E: DNA331489, NP\_066267.1, 206385\_s\_at  
 Figure 1761: PRO86532  
 Figure 1762: DNA326727, NP\_001527.1, 206445\_s\_at  
 Figure 1763: PRO83069  
 Figure 1764A-B: DNA271891, NP\_055766.1, 206448\_at  
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 Figure 1766: DNA153751, NP\_005942.1, 206461\_x\_at  
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 Figure 1768: DNA88203, NP\_055022.1, 206485\_at  
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 Figure 1770: DNA288243, NP\_002277.3, 206486\_at  
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 Figure 1772: DNA269850, NP\_002003.1, 206492\_at  
 Figure 1773: PRO58251  
 Figure 1774: DNA270444, NP\_004824.1, 206513\_at  
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 Figure 1780: DNA331490, OAS2, 206553\_at  
 Figure 1781: PRO69656  
 Figure 1782: DNA227540, NP\_003036.1, 206566\_at  
 Figure 1783: PRO38003  
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 Figure 1785: PRO19671  
 Figure 1786: DNA329172, NP\_005254.1, 206589\_at  
 Figure 1787: PRO84796  
 Figure 1788: DNA103451, NP\_003846.1, 206618\_at  
 Figure 1789: PRO4778

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Figure 1791: PRO38172  
Figure 1792: DNA331491, NP\_004891.2, 206632\_s\_at  
Figure 1793: PRO62308  
Figure 1794: DNA331492, BCL2L1, 206665\_s\_at  
Figure 1795: PRO83141  
Figure 1796: DNA88374, NP\_002095.1, 206666\_s\_at  
Figure 1797: PRO2768  
Figure 1798: DNA330105, HUMNCAK, 206676\_s\_at  
Figure 1799: PRO85372  
Figure 1800: DNA328590, C6orf32, 206707\_x\_at  
Figure 1801: PRO84375  
Figure 1802: DNA330106, NP\_003646.1, 206724\_s\_at  
Figure 1803: PRO85373  
Figure 1804A-B: DNA88191, NP\_001234.1, 206729\_s\_at  
Figure 1805: PRO2691  
Figure 1806A-B: DNA88650, NP\_005807.1, 206761\_s\_at  
Figure 1807: PRO2460  
Figure 1808: DNA226427, NP\_002251.1, 206785\_s\_at  
Figure 1809: PRO36890  
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Figure 1820: DNA331493, CCR2, 206978\_s\_at  
Figure 1821: PRO84690  
Figure 1822: DNA188346, NP\_001450.1, 206980\_s\_at  
Figure 1823: PRO21766  
Figure 1824A-B: DNA227659, NP\_000570.1, 206991\_s\_at  
Figure 1825: PRO38122  
Figure 1826A-B: DNA227750, NP\_001550.1, 206999\_s\_at  
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Figure 1828: DNA329903, PPP3CC, 207000\_s\_at  
Figure 1829: PRO85220  
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Figure 1831: PRO85374  
Figure 1832: DNA331494, PLAGL1, 207002\_s\_at  
Figure 1833: PRO62736  
Figure 1834: DNA331495, HUMBCL2B, 207005\_s\_at  
Figure 1835: PRO86533  
Figure 1836: DNA330110, HUMK10A, 207023\_x\_at  
Figure 1837: PRO85375  
Figure 1838: DNA225550, NP\_003844.1, 207072\_s\_at  
Figure 1839: PRO36013  
Figure 1840: DNA273159, NP\_005457.1, 207078\_s\_at  
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Figure 1844: DNA218655, NP\_000585.1, 207113\_s\_at  
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Figure 1846: DNA330111, NP\_002615.2, 207132\_x\_at  
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Figure 1848: DNA330112, NP\_444504.1, 207153\_s\_at  
Figure 1849: PRO61610  
Figure 1850: DNA103418, NP\_036616.1, 207165\_s\_at  
Figure 1851: PRO4746  
Figure 1852: DNA330113, NP\_203124.1, 207181\_s\_at  
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Figure 1860A-B: DNA330115, NP\_077739.1, 207324\_s\_at  
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Figure 1862A-B: DNA226536, NP\_003225.1, 207332\_s\_at  
Figure 1863: PRO36999  
Figure 1864: DNA331497, LTB, 207339\_s\_at  
Figure 1865: PRO11604  
Figure 1866: DNA330117, NP\_003966.1, 207351\_s\_at  
Figure 1867: PRO85379  
Figure 1868: DNA330118, NP\_036389.2, 207361\_s\_at  
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Figure 1876: DNA36718, NP\_000563.1, 207433\_s\_at  
Figure 1877: PRO73  
Figure 1878A-B: DNA330119, NP\_060189.2, 207474\_s\_at  
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Figure 1881: PRO84381  
Figure 1882: DNA328597, ATP5G3, 207508\_s\_at  
Figure 1883: PRO84381  
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Figure 1885: PRO51107  
Figure 1886A-B: DNA256059, ATP2A3, 207522\_s\_at  
Figure 1887: PRO51107  
Figure 1888: DNA304473, NP\_001552.2, 207536\_s\_at  
Figure 1889: PRO2023  
Figure 1890: DNA325454, NP\_003637.1, 207556\_s\_at  
Figure 1891: PRO81977  
Figure 1892: DNA328601, NP\_056490.1, 207574\_s\_at  
Figure 1893: PRO84384  
Figure 1894A-B: DNA330120, FLJ10971, 207606\_s\_at  
Figure 1895: PRO85382

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 Figure 1898: DNA331498, TANK, 207616\_s\_at  
 Figure 1899: PRO86535  
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 Figure 1901: PRO36800  
 Figure 1902: DNA227606, NP\_001872.2, 207630\_s\_at  
 Figure 1903: PRO38069  
 Figure 1904: DNA196426, NP\_037440.1, 207651\_at  
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 Figure 1906: DNA328554, NP\_038202.1, 207677\_s\_at  
 Figure 1907: PRO84354  
 Figure 1908A-B: DNA226405, NP\_006525.1, 207700\_s\_at  
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 Figure 1910: DNA329064, NP\_060301.1, 207735\_at  
 Figure 1911: PRO84724  
 Figure 1912: DNA329020, NUP62, 207740\_s\_at  
 Figure 1913: PRO84695  
 Figure 1914: DNA325654, NP\_054752.1, 207761\_s\_at  
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 Figure 1916A-B: DNA329179, NP\_056958.1, 207785\_s\_at  
 Figure 1917: PRO84802  
 Figure 1918: DNA227494, NP\_002158.1, 207826\_s\_at  
 Figure 1919: PRO37957  
 Figure 1920A-C: DNA331499, NP\_057427.2, 207828\_s\_at  
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 Figure 1931: PRO34287  
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 Figure 1933: PRO34447  
 Figure 1934: DNA330125, NP\_002729.2, 207957\_s\_at  
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 Figure 1936A-B: DNA226290, NP\_036333.1, 207966\_s\_at  
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 Figure 1941: PRO85386  
 Figure 1942: DNA329184, CITED2, 207980\_s\_at  
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 Figure 1944A-C: DNA254145, NP\_004329.1, 207996\_s\_at  
 Figure 1945: PRO49260
- Figure 1946: DNA275286, NP\_009205.1, 208002\_s\_at  
 Figure 1947: PRO62967  
 Figure 1948: DNA288217, NP\_002101.1, 208018\_s\_at  
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 Figure 1950: DNA227224, NP\_060877.1, 208029\_s\_at  
 Figure 1951: PRO37687  
 Figure 1952A-B: DNA188492, NAB1, 208047\_s\_at  
 Figure 1953: PRO22070  
 Figure 1954: DNA330127, NP\_006442.2, 208051\_s\_at  
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 Figure 1957: PRO84390  
 Figure 1958A-C: DNA331500, NP\_003307.2, 208073\_x\_at  
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 Figure 1960A-B: DNA328312, NP\_110378.1, 208078\_s\_at  
 Figure 1961: PRO84180  
 Figure 1962: DNA331501, STK6, 208079\_s\_at  
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 Figure 1967: PRO85389  
 Figure 1968: DNA325329, NP\_004719.1, 208152\_s\_at  
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 Figure 1972A-E: DNA330130, HSTITIN, 208195\_at  
 Figure 1973: DNA328611, RASGRP2, 208206\_s\_at  
 Figure 1974: PRO84393  
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 Figure 1977A-D: DNA331502, NP\_000050.1, 208368\_s\_at  
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 Figure 1983: DNA327690, NP\_004022.1, 208436\_s\_at  
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Figure 1998: PRO85397  
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Figure 2005: DNA325912, ACTN1, 208637.x.at  
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Figure 2020: PRO12135  
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Figure 2024A-B: DNA328619, BC001188, 208691.at  
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Figure 2026: DNA287189, NP\_002038.1, 208693.s.at  
Figure 2027: PRO69475  
Figure 2028: DNA330140, AF275798, 208696.at  
Figure 2029: PRO85399  
Figure 2030A-C: DNA331508, 198777.9, 208707.at  
Figure 2031: PRO86543  
Figure 2032: DNA97298, NP\_003899.1, 208726.s.at  
Figure 2033: PRO3645  
Figure 2034: DNA330142, BC003564, 208737.at  
Figure 2035: PRO85401  
Figure 2036: DNA331509, 1138554.23, 208740.at  
Figure 2037: PRO86544  
Figure 2038: DNA328591, HSP105B, 208744.x.at  
Figure 2039: PRO84376  
Figure 2040: DNA287285, NP\_005794.1, 208748.s.at  
Figure 2041: PRO69556  
Figure 2042: DNA324217, ATIC, 208758.at  
Figure 2043: PRO80908  
Figure 2044: DNA327696, AF228339, 208763.s.at  
Figure 2045: PRO83679  
Figure 2046A-B: DNA331510, 1298307.1, 208776.at  
Figure 2047: PRO86545  
Figure 2048: DNA287427, NP\_002806.1, 208777.s.at  
Figure 2049: PRO69684  
Figure 2050: DNA287219, NP\_110379.1, 208778.s.at  
Figure 2051: PRO69498  
Figure 2052: DNA329189, NP\_009139.1, 208787.at  
Figure 2053: PRO4911  
Figure 2054: DNA238565, NP\_005907.2, 208795.s.at  
Figure 2055: PRO39210  
Figure 2056: DNA330145, NP\_002788.1, 208799.at  
Figure 2057: PRO84403  
Figure 2058: DNA331511, HSMPIO, 208805.at  
Figure 2059A-C: DNA331512, 1397486.26, 208806.at  
Figure 2060: PRO86547  
Figure 2061A-B: DNA330147, HSU91543, 208807.s.at  
Figure 2062: PRO85405  
Figure 2063: DNA324531, NP\_002120.1, 208808.s.at  
Figure 2064: PRO81185  
Figure 2065: DNA273521, NP\_002070.1, 208813.at  
Figure 2066: PRO61502  
Figure 2067A-B: DNA330148, AB020636, 208838.at  
Figure 2068A-B: DNA330149, HSM801778, 208839.s.at  
Figure 2069: PRO82209  
Figure 2070: DNA227874, NP\_003320.1, 208864.s.at  
Figure 2071: PRO38337  
Figure 2072: DNA328624, BC003562, 208891.at  
Figure 2073: PRO59076  
Figure 2074: DNA331513, DUSP6, 208892.s.at  
Figure 2075: PRO84404  
Figure 2076: DNA331330, BC005047, 208893.s.at  
Figure 2077: PRO82215  
Figure 2078: DNA329221, NP\_061984.1, 208894.at  
Figure 2079: PRO4555  
Figure 2080A-B: DNA329007, NP\_003277.1, 208900.s.at  
Figure 2081: PRO37029  
Figure 2082A-B: DNA329007, TOP1, 208901.s.at  
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Figure 2084: DNA327700, BC015130, 208905.at  
Figure 2085: PRO83683  
Figure 2086: DNA327701, NP\_001203.1, 208910.s.at  
Figure 2087: PRO82667  
Figure 2088: DNA281442, NP\_149124.1, 208912.s.at  
Figure 2089: PRO66281  
Figure 2090A-B: DNA330151, AB029003, 208914.at  
Figure 2091: DNA325473, NP\_006353.2, 208922.s.at  
Figure 2092: PRO81996  
Figure 2093: DNA329552, NP\_063948.1, 208925.at  
Figure 2094: PRO85097  
Figure 2095: DNA326233, NP\_000968.2, 208929.x.at  
Figure 2096: PRO82645  
Figure 2097: DNA327702, NP\_006490.2, 208934.s.at  
Figure 2098: PRO83684  
Figure 2099: DNA330152, NP\_001939.1, 208956.x.at  
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Figure 2101: DNA290261, NP\_001291.2, 208960.s.at  
Figure 2102: PRO70387  
Figure 2103A-B: DNA325478, NP\_037534.2,

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 Figure 2105: DNA327661, IFI16, 208965\_s\_at  
 Figure 2106: PRO83652  
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 Figure 2108: PRO58665  
 Figure 2109: DNA326343, KPNB1, 208974\_x\_at  
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 Figure 2111A-B: DNA330153, HUMIMP90A,  
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 Figure 2112: PRO82739  
 Figure 2113: DNA328629, NP\_006079.1, 208977\_x\_at  
 Figure 2114: PRO84407  
 Figure 2115: DNA330154, HUMPECAM27,  
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 Figure 2116: DNA330155, 7692317.2, 208982\_at  
 Figure 2117: PRO85407  
 Figure 2118: DNA330156, NP\_003749.1, 208985\_s\_at  
 Figure 2119: PRO85408  
 Figure 2120: DNA331514, STAT3, 208992\_s\_at  
 Figure 2121: PRO86548  
 Figure 2122: DNA227552, NP\_003346.2, 208997\_s\_at  
 Figure 2123: PRO38015  
 Figure 2124: DNA227552, UCP2, 208998\_at  
 Figure 2125: PRO38015  
 Figure 2126: DNA328630, NP\_036293.1, 209004\_s\_at  
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 Figure 2128: DNA331515, FBXL5, 209005\_at  
 Figure 2129: PRO86549  
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 Figure 2131: PRO84409  
 Figure 2132: DNA331516, DNAJB6, 209015\_s\_at  
 Figure 2133: PRO83680  
 Figure 2134: DNA328633, NP\_004784.2, 209017\_s\_at  
 Figure 2135: PRO84411  
 Figure 2136: DNA330158, NP\_057554.4, 209020\_at  
 Figure 2137: PRO85410  
 Figure 2138: DNA327851, NP\_006363.2, 209024\_s\_at  
 Figure 2139: PRO83795  
 Figure 2140: DNA328635, BC020946, 209026\_x\_at  
 Figure 2141: PRO84413  
 Figure 2142: DNA331517, NP\_004150.1, 209040\_s\_at  
 Figure 2143: PRO69506  
 Figure 2144A-C: DNA328637, HSA7042, 209052\_s\_at  
 Figure 2145: PRO81109  
 Figure 2146A-B: DNA331518, AF330040,  
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 Figure 2148A-B: DNA226405, NCOA3, 209060\_x\_at  
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 Figure 2152: DNA330160, NP\_006285.1, 209066\_x\_at  
 Figure 2153: PRO85412  
 Figure 2154: DNA329194, NP\_112740.1, 209068\_at  
 Figure 2155: PRO84814  
 Figure 2156A-B: DNA330161, NP\_085059.1,  
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 Figure 2158: DNA330162, NP\_057093.1, 209091\_s\_at  
 Figure 2159: PRO85414  
 Figure 2160: DNA330163, NP\_060308.1, 209104\_s\_at  
 Figure 2161: PRO85415  
 Figure 2162: DNA330164, NP\_004752.1, 209110\_s\_at  
 Figure 2163: PRO85416  
 Figure 2164: DNA327709, NP\_000509.1, 209116\_x\_at  
 Figure 2165: PRO83690  
 Figure 2166: DNA288254, NP\_006000.2, 209118\_s\_at  
 Figure 2167: PRO69536  
 Figure 2168: DNA325163, NP\_001113.1, 209122\_at  
 Figure 2169: PRO81730  
 Figure 2170: DNA330165, BC015833, 209138\_x\_at  
 Figure 2171: PRO85417  
 Figure 2172: DNA327713, BC010653, 209146\_at  
 Figure 2173: PRO37975  
 Figure 2174: DNA325285, AKR1C3, 209160\_at  
 Figure 2175: PRO81832  
 Figure 2176: DNA330166, BC001588, 209161\_at  
 Figure 2177: PRO85418  
 Figure 2178: DNA271722, NP\_004688.1, 209162\_s\_at  
 Figure 2179: PRO60006  
 Figure 2180: DNA330167, CAB43224.1, 209177\_at  
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 Figure 2183: PRO84418  
 Figure 2184: DNA331331, AF161416, 209185\_s\_at  
 Figure 2185A-B: DNA328643, HUMHK1A,  
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 Figure 2187: DNA189700, NP\_005243.1, 209189\_at  
 Figure 2188: PRO25619  
 Figure 2189: DNA324766, NP\_005443.2, 209196\_at  
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 Figure 2191: DNA226176, NP\_003458.1, 209201\_x\_at  
 Figure 2192: PRO36639  
 Figure 2193: DNA326267, NP\_004861.1, 209208\_at  
 Figure 2194: PRO82674  
 Figure 2195: DNA326891, NP\_001748.1, 209213\_at  
 Figure 2196: PRO83212  
 Figure 2197: DNA227483, NP\_003120.1, 209218\_at  
 Figure 2198: PRO37946  
 Figure 2199: DNA330168, NP\_006322.1, 209233\_at  
 Figure 2200: PRO85420  
 Figure 2201: DNA328649, NP\_116093.1, 209251\_x\_at  
 Figure 2202: PRO84424  
 Figure 2203: DNA255255, NP\_071437.1, 209267\_s\_at  
 Figure 2204: PRO50332  
 Figure 2205A-B: DNA188492, AF045451, 209272\_at  
 Figure 2206: PRO22070  
 Figure 2207A-B: DNA226827, NP\_001673.1,

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 Figure 2209: DNA328601, GADD45B, 209304\_x\_at  
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 Figure 2211: DNA328651, AF087853, 209305\_s\_at  
 Figure 2212: PRO82889  
 Figure 2213: DNA151780, NP\_006611.1, 209314\_s\_at  
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 Figure 2215: DNA330169, NP\_006709.1, 209318\_x\_at  
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 Figure 2217: DNA275106, HSU76248, 209339\_at  
 Figure 2218: PRO62821  
 Figure 2219: DNA269630, NP\_003281.1, 209344\_at  
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 Figure 2223: DNA330170, AF109161, 209357\_at  
 Figure 2224: PRO84807  
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 Figure 2228: PRO85421  
 Figure 2229: DNA330172, BC009529, 209377\_s\_at  
 Figure 2230: PRO85422  
 Figure 2231: DNA330173, HUMAUTOTAX,  
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 Figure 2232: PRO85423  
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 Figure 2235: DNA330175, NP\_006836.1, 209408\_at  
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 Figure 2237A-B: DNA271241, HSU61500, 209412\_at  
 Figure 2238: PRO59556  
 Figure 2239: DNA330176, AAB61703.1, 209417\_s\_at  
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 Figure 2242: PRO36230  
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 Figure 2244: PRO10283  
 Figure 2245: DNA273076, HSU59863, 209451\_at  
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 Figure 2258: PRO81503  
 Figure 2259: DNA330180, NP\_009149.2, 209510\_at  
 Figure 2260: PRO85428  
 Figure 2261: DNA274027, RAB27A, 209514\_s\_at  
 Figure 2262: PRO61971  
 Figure 2263: DNA274027, HSU38654, 209515\_s\_at  
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 Figure 2265: DNA272213, NP\_002477.1, 209520\_s\_at  
 Figure 2266: PRO60475  
 Figure 2267: DNA330181, HSM802358, 209523\_at  
 Figure 2268: DNA328663, NP\_057157.1, 209524\_at  
 Figure 2269: PRO36183  
 Figure 2270: DNA330182, PLAA, 209533\_s\_at  
 Figure 2271: PRO85430  
 Figure 2272: DNA330183, AF181265, 209536\_s\_at  
 Figure 2273: DNA327724, AF323542S7, 209546\_s\_at  
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 Figure 2276: DNA330184, BC022475, 209566\_at  
 Figure 2277: PRO85432  
 Figure 2278: DNA290251, NP\_055207.1, 209569\_x\_at  
 Figure 2279: PRO70367  
 Figure 2280: DNA329202, BC001745, 209570\_s\_at  
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 Figure 2282: DNA329203, NP\_003788.1, 209572\_s\_at  
 Figure 2283: PRO84819  
 Figure 2284: DNA304797, NP\_005935.3, 209583\_s\_at  
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 Figure 2288: DNA270689, NP\_002042.1, 209604\_s\_at  
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 Figure 2293: PRO70011  
 Figure 2294: DNA330185, NP\_071415.1, 209624\_s\_at  
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 Figure 2297: PRO84382  
 Figure 2298: DNA330186, NP\_004327.1, 209642\_at  
 Figure 2299: PRO85434  
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 Figure 2302: DNA330188, NP\_004356.1, 209662\_at  
 Figure 2303: PRO85436  
 Figure 2304: DNA323856, PAI-RBP1, 209669\_s\_at  
 Figure 2305: PRO80599  
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 Figure 2308: DNA193881, AAF15129.1, 209681\_at  
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 Figure 2333: DNA327731, NP\_003302.1, 209803\_s\_at  
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 Figure 2363: DNA330152, DUT, 209932\_s\_at  
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 Figure 2396A-B: DNA328685, NP\_127497.1,  
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Figure 2470: DNA331529, LAIR1, 210644\_s\_at  
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Figure 2472A-C: DNA330214, HUMTPRD, 210645\_s\_at  
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Figure 2489: DNA329219, BC000385, 210844\_x\_at  
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Figure 2492: PRO86255  
Figure 2493: DNA188234, NP\_000630.1, 210865\_at  
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Figure 2495: DNA331531, PFDN5, 210908\_s\_at  
Figure 2496: PRO86560  
Figure 2497: DNA330217, AF043183, 210915\_x\_at  
Figure 2498: PRO85458  
Figure 2499: DNA274326, NP\_003079.1, 210933\_s\_at  
Figure 2500: PRO62244  
Figure 2501: DNA329317, NP\_057353.1, 210948\_s\_at  
Figure 2502: PRO81157  
Figure 2503: DNA331532, AF125393, 210951\_x\_at  
Figure 2504: PRO86561  
Figure 2505: DNA330218, HUMTCAXA, 210972\_x\_at  
Figure 2506: DNA273236, NP\_004306.1, 210980\_s\_at  
Figure 2507: PRO61263  
Figure 2508: DNA269888, NP\_002073.1, 210981\_s\_at  
Figure 2509: PRO58286  
Figure 2510: DNA329221, HLA-DRA, 210982\_s\_at  
Figure 2511: PRO4555  
Figure 2512: DNA238565, MCM7, 210983\_s\_at  
Figure 2513: PRO39210  
Figure 2514: DNA326239, YWHAE, 210996\_s\_at  
Figure 2515: PRO39530  
Figure 2516A-B: DNA330219, NP\_150241.1, 211013\_x\_at  
Figure 2517: PRO85459  
Figure 2518: DNA327699, AB023420, 211015\_s\_at  
Figure 2519: PRO83682  
Figure 2520: DNA288254, TUBA3, 211058\_x\_at  
Figure 2521: PRO69536  
Figure 2522: DNA329992, MGAT2, 211061\_s\_at  
Figure 2523: PRO59267  
Figure 2524: DNA324171, NP\_065438.1, 211070\_x\_at  
Figure 2525: PRO60753

- Figure 2526: DNA330220, NP\_006809.1, 211071\_s\_at  
 Figure 2527: PRO60769  
 Figure 2528: DNA287198, K-ALPHA-1, 211072\_x\_at  
 Figure 2529: PRO69484  
 Figure 2530: DNA254470, NEK2, 211080\_s\_at  
 Figure 2531: PRO49578  
 Figure 2532: DNA196432, AF064804, 211106\_at  
 Figure 2533: PRO24928  
 Figure 2534: DNA330202, CXCL11, 211122\_s\_at  
 Figure 2535: PRO19838  
 Figure 2536: DNA304765, HUMTCRGAD,  
 211144\_x\_at  
 Figure 2537: PRO71178  
 Figure 2538: DNA327752, HSDHACTYL,  
 211150\_s\_at  
 Figure 2539A-B: DNA328700, SCD, 211162\_x\_at  
 Figure 2540: PRO84464  
 Figure 2541: DNA330221, NP\_056071.1, 211207\_s\_at  
 Figure 2542: PRO85460  
 Figure 2543: DNA330222, NP\_003848.1, 211226\_at  
 Figure 2544: PRO45958  
 Figure 2545: DNA218278, IL2RA, 211269\_s\_at  
 Figure 2546: PRO34330  
 Figure 2547: DNA151022, DGKA, 211272\_s\_at  
 Figure 2548: PRO12096  
 Figure 2549: DNA330223, NP\_001790.1, 211297\_s\_at  
 Figure 2550: PRO49730  
 Figure 2551A-C: DNA328811, ITPR1, 211323\_s\_at  
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 Figure 2553: DNA188234, TNFSF6, 211333\_s\_at  
 Figure 2554: PRO21942  
 Figure 2555: DNA103395, HSU80737, 211352\_s\_at  
 Figure 2556: PRO4723  
 Figure 2557A-B: DNA275066, NP\_000170.1,  
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 Figure 2558: PRO62786  
 Figure 2559: DNA327755, NP\_115957.1, 211458\_s\_at  
 Figure 2560: PRO83725  
 Figure 2561: DNA93439, CXCR6, 211469\_s\_at  
 Figure 2562: PRO4515  
 Figure 2563: DNA330175, KNSL6, 211519\_s\_at  
 Figure 2564: PRO59681  
 Figure 2565: DNA327756, NP\_068814.2, 211538\_s\_at  
 Figure 2566: PRO83726  
 Figure 2567: DNA269888, GPRK6, 211543\_s\_at  
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 Figure 2572: PRO85454  
 Figure 2573: DNA330224, HUMNCA, 211657\_at  
 Figure 2574: PRO85461  
 Figure 2575: DNA327709, HBB, 211696\_x\_at  
 Figure 2576: PRO83690  
 Figure 2577: DNA331533, PPARG, 211699\_x\_at  
 Figure 2578: PRO86562
- Figure 2579: DNA331534, AF116616, 211708\_s\_at  
 Figure 2580: DNA226342, PTEN, 211711\_s\_at  
 Figure 2581: PRO36805  
 Figure 2582: DNA328706, BC021909, 211714\_x\_at  
 Figure 2583: PRO10347  
 Figure 2584: DNA88307, NP\_001992.1, 211734\_s\_at  
 Figure 2585: PRO2280  
 Figure 2586: DNA329225, EVI2B, 211742\_s\_at  
 Figure 2587: PRO84833  
 Figure 2588: DNA331535, AF105974, 211745\_x\_at  
 Figure 2589: PRO3629  
 Figure 2590: DNA328649, TUBA6, 211750\_x\_at  
 Figure 2591: PRO84424  
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 Figure 2593: PRO49824  
 Figure 2594: DNA330225, NP\_115712.1, 211767\_at  
 Figure 2595: PRO85462  
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 Figure 2599: PRO2023  
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 Figure 2605: PRO86563  
 Figure 2606: DNA331537, CCNE2, 211814\_s\_at  
 Figure 2607: PRO59418  
 Figure 2608A-B: DNA331342, DEFCAP, 211822\_s\_at  
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 Figure 2611: PRO86423  
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 Figure 2615: PRO86565  
 Figure 2616A-B: DNA188192, CD28, 211856\_x\_at  
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 Figure 2618: DNA331540, AF222343, 211861\_x\_at  
 Figure 2619: PRO86566  
 Figure 2620: DNA330228, HUMTCRAZ, 211902\_x\_at  
 Figure 2621: PRO85465  
 Figure 2622: DNA226176, CXCR4, 211919\_s\_at  
 Figure 2623: PRO36639  
 Figure 2624: DNA272286, CAT, 211922\_s\_at  
 Figure 2625: PRO60544  
 Figure 2626: DNA330229, BC011915, 211926\_s\_at  
 Figure 2627: PRO85466  
 Figure 2628: DNA226254, NP\_001408.1, 211937\_at  
 Figure 2629: PRO36717  
 Figure 2630: DNA330230, NP\_060977.1, 211938\_at  
 Figure 2631: PRO85467  
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 Figure 2633: PRO81851

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Figure 2635: DNA328437, NP\_005792.1, 211956\_s\_at  
Figure 2636: PRO84271  
Figure 2637: DNA325941, NP\_005339.1, 211969\_at  
Figure 2638: PRO82388  
Figure 2639: DNA287194, AAA60258.1, 211974\_x\_at  
Figure 2640: PRO69480  
Figure 2641A-C: DNA331541, 1390535.1, 211986\_at  
Figure 2642: PRO86567  
Figure 2643: DNA330232, NP\_291032.1, 211991\_s\_at  
Figure 2644: PRO85469  
Figure 2645: DNA330233, AF218029, 211999\_at  
Figure 2646: PRO11403  
Figure 2647: DNA287433, NP\_006810.1, 212009\_s\_at  
Figure 2648: PRO69690  
Figure 2649A-D: DNA103461, MKI67, 212020\_s\_at  
Figure 2650: PRO4788  
Figure 2651A-D: DNA226463, HSMKI67A, 212021\_s\_at  
Figure 2652: PRO36926  
Figure 2653A-D: DNA103461, HSMKI67, 212022\_s\_at  
Figure 2654: PRO4788  
Figure 2655A-D: DNA226463, DNA226463, 212023\_s\_at  
Figure 2656: PRO36926  
Figure 2657: DNA275447, HSMEMA, 212037\_at  
Figure 2658: PRO63095  
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Figure 2660: PRO4710  
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Figure 2662: PRO85470  
Figure 2663: DNA328709, BC004151, 212048\_s\_at  
Figure 2664: PRO37676  
Figure 2665A-B: DNA330235, BAA20790.1, 212061\_at  
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Figure 2668: PRO85472  
Figure 2669: DNA154139, DNA154139, 212099\_at  
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Figure 2672A-B: DNA150956, BAA06685.1, 212110\_at  
Figure 2673: PRO12560  
Figure 2674: DNA328711, AK023154, 212115\_at  
Figure 2675: PRO84468  
Figure 2676: DNA219225, NP\_002874.1, 212125\_at  
Figure 2677: PRO34531  
Figure 2678: DNA330238, BC019676, 212127\_at  
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Figure 2681: DNA330239, AK027643, 212131\_at  
Figure 2682: PRO85474  
Figure 2683: DNA330240, CAA52801.1, 212141\_at  
Figure 2684: PRO85475  
Figure 2685: DNA330240, HSP1CDC21, 212142\_at  
Figure 2686A-B: DNA150829, AB014568, 212144\_at  
Figure 2687: DNA329602, AK2, 212175\_s\_at  
Figure 2688: PRO85133  
Figure 2689: DNA330241, AF314185, 212176\_at  
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Figure 2691: DNA330242, BC007034, 212185\_x\_at  
Figure 2692: PRO85477  
Figure 2693: DNA330243, BC015663, 212190\_at  
Figure 2694: PRO2584  
Figure 2695: DNA326233, RPL13, 212191\_x\_at  
Figure 2696: PRO82645  
Figure 2697A-C: DNA330244, 253946.17, 212196\_at  
Figure 2698: PRO85478  
Figure 2699A-B: DNA330245, 230497.7, 212206\_s\_at  
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Figure 2701: DNA331543, BC008710, 212227\_x\_at  
Figure 2702: PRO84271  
Figure 2703: DNA327770, 1384008.4, 212239\_at  
Figure 2704: PRO83736  
Figure 2705: DNA151120, DNA151120, 212240\_s\_at  
Figure 2706: PRO12179  
Figure 2707: DNA330246, AF326773, 212241\_at  
Figure 2708A-B: DNA329229, 1345070.7, 212249\_at  
Figure 2709: PRO84835  
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Figure 2711: PRO84805  
Figure 2712: DNA331544, BC018823, 212266\_s\_at  
Figure 2713: PRO86569  
Figure 2714: DNA327771, NP\_109591.1, 212268\_at  
Figure 2715: PRO83737  
Figure 2716: DNA326463, NP\_000976.1, 212270\_x\_at  
Figure 2717: PRO82846  
Figure 2718: DNA150980, HUMMAC30X, 212279\_at  
Figure 2719: DNA150980, DNA150980, 212281\_s\_at  
Figure 2720: PRO12566  
Figure 2721: DNA253017, DNA253017, 212282\_at  
Figure 2722: PRO48926  
Figure 2723: DNA328719, BC012895, 212295\_s\_at  
Figure 2724: PRO84475  
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Figure 2726: PRO59425  
Figure 2727: DNA207620, DNA207620, 212300\_at  
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Figure 2729: PRO85481  
Figure 2730: DNA330248, BC019924, 212320\_at  
Figure 2731: PRO10347  
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Figure 2733: PRO6323  
Figure 2734A-B: DNA124122, NP\_005602.2, 212332\_at  
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Figure 2736: DNA287190, CAB43217.1, 212333\_at  
Figure 2737: PRO69476

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 Figure 2739: PRO85457  
 Figure 2740A-B: DNA327773, BAA25456.1, 212366\_at  
 Figure 2741: PRO83739  
 Figure 2742A-C: DNA330249, AAA99177.1, 212372\_at  
 Figure 2743: PRO85482  
 Figure 2744: DNA329231, NP\_000810.1, 212378\_at  
 Figure 2745: PRO84837  
 Figure 2746: DNA329231, GART, 212379\_at  
 Figure 2747: PRO84837  
 Figure 2748A-B: DNA150950, HUMKIAAH, 212396\_s\_at  
 Figure 2749A-B: DNA328549, NP\_002897.1, 212397\_at  
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 Figure 2751A-B: DNA328549, RDX, 212398\_at  
 Figure 2752: PRO84350  
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 Figure 2754: PRO11708  
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 Figure 2758: PRO49923  
 Figure 2759: DNA330251, NP\_059965.1, 212430\_at  
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 Figure 2762: PRO83740  
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 Figure 2765: DNA269630, TPM4, 212481\_s\_at  
 Figure 2766: PRO58042  
 Figure 2767: DNA330253, BC007665, 212493\_s\_at  
 Figure 2768: PRO85486  
 Figure 2769: DNA330254, AK024029, 212508\_at  
 Figure 2770: PRO85487  
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 Figure 2773: DNA329233, 383512.16, 212527\_at  
 Figure 2774: PRO84839  
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 Figure 2776: PRO36504  
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 Figure 2778: PRO58280  
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 Figure 2780: PRO84490  
 Figure 2781A-B: DNA330255, AK025499, 212561\_at  
 Figure 2782: PRO85488  
 Figure 2783: DNA225632, NP\_002037.2, 212581\_x\_at  
 Figure 2784: PRO36095  
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 Figure 2786: PRO84841
- Figure 2787A-B: DNA331545, AB040884, 212585\_at  
 Figure 2788: DNA275100, DNA275100, 212589\_at  
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 Figure 2790: PRO81145  
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 Figure 2792: PRO85489  
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 Figure 2794: PRO85490  
 Figure 2795A-B: DNA330258, AB006624, 212621\_at  
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 Figure 2799: PRO38556  
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 Figure 2801A-B: DNA327778, AB011154, 212675\_s\_at  
 Figure 2802: DNA273465, DNA273465, 212677\_s\_at  
 Figure 2803: DNA328744, AF318364, 212680\_x\_at  
 Figure 2804: PRO84496  
 Figure 2805A-B: DNA329901, AB007915, 212683\_at  
 Figure 2806A-B: DNA269508, AB011110, 212706\_at  
 Figure 2807A-B: DNA331546, 332730.12, 212714\_at  
 Figure 2808: PRO86570  
 Figure 2809: DNA331547, BC010994, 212734\_x\_at  
 Figure 2810: PRO82645  
 Figure 2811: DNA329906, BC007848, 212738\_at  
 Figure 2812: PRO85223  
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 Figure 2814: PRO85492  
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 Figure 2820: PRO86571  
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 Figure 2822: PRO12260  
 Figure 2823A-B: DNA331549, BAA07892.2, 212832\_s\_at  
 Figure 2824: PRO86572  
 Figure 2825: DNA271714, BAA05039.1, 212836\_at  
 Figure 2826: PRO59998  
 Figure 2827: DNA331550, AAA59587.1, 212859\_x\_at  
 Figure 2828: PRO6386  
 Figure 2829A-B: DNA328753, BAA13212.1, 212873\_at  
 Figure 2830: PRO84502  
 Figure 2831: DNA330265, NP\_056436.1, 212886\_at  
 Figure 2832: PRO85495  
 Figure 2833A-B: DNA271215, BAA24380.1, 212892\_at  
 Figure 2834: PRO59530

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 Figure 2836: PRO85496  
 Figure 2837A-B: DNA271137, AB014589, 212905\_at  
 Figure 2838: DNA271630, DNA271630, 212907\_at  
 Figure 2839: DNA330267, 235076.14, 212914\_at  
 Figure 2840: PRO85497  
 Figure 2841: DNA330268, BC009116, 212928\_at  
 Figure 2842: PRO85498  
 Figure 2843: DNA331551, BC013078, 212933\_x\_at  
 Figure 2844: PRO82645  
 Figure 2845A-B: DNA330269, BC020584, 212936\_at  
 Figure 2846: PRO23868  
 Figure 2847: DNA330270, HUMORF007, 212949\_at  
 Figure 2848A-B: DNA331552, PAM, 212958\_x\_at  
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 Figure 2851: DNA330271, 399773.20, 212980\_at  
 Figure 2852: PRO85500  
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 Figure 2861: PRO86574  
 Figure 2862A-B: DNA253815, BAA20833.2, 213035\_at  
 Figure 2863: PRO49218  
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 Figure 2872: DNA270466, HUMG6PD, 213093\_at  
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 Figure 2878: PRO69509  
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 Figure 2880: PRO85506  
 Figure 2881: DNA228053, DNA228053, 213156\_at  
 Figure 2882: DNA151370, DNA151370, 213158\_at  
 Figure 2883: PRO11747  
 Figure 2884: DNA106374, DNA106374, 213164\_at  
 Figure 2885A-B: DNA330278, BAA13216.1, 213174\_at  
 Figure 2886: PRO85507  
 Figure 2887: DNA330279, AF043182, 213193\_x\_at  
 Figure 2888: PRO85508  
 Figure 2889: DNA227909, NP\_005024.1, 213226\_at  
 Figure 2890: PRO38372  
 Figure 2891A-B: DNA330280, BAA83045.2, 213254\_at  
 Figure 2892: PRO85509  
 Figure 2893A-B: DNA328761, BAA82991.1, 213280\_at  
 Figure 2894: PRO84509  
 Figure 2895: DNA331555, BC009874, 213281\_at  
 Figure 2896A-B: DNA274945, HSACKI10, 213287\_s\_at  
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 Figure 2898: PRO54720  
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 Figure 2900: PRO84217  
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 Figure 2902: PRO84850  
 Figure 2903A-B: DNA255273, BAA83044.1, 213309\_at  
 Figure 2904: PRO50349  
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 Figure 2906A-B: DNA331355, AAG24545.1, 213330\_s\_at  
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 Figure 2908A-B: DNA330281, AB058688, 213341\_at  
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 Figure 2910: PRO83753  
 Figure 2911: DNA287176, AB025254, 213361\_at  
 Figure 2912: DNA327790, 1448999.3, 213364\_s\_at  
 Figure 2913: PRO83754  
 Figure 2914A-B: DNA330282, 217860.13, 213376\_at  
 Figure 2915: PRO85510  
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 Figure 2919: PRO85512  
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 Figure 2921: PRO36095  
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 Figure 2932: DNA227483, SQLE, 213562\_s\_at  
 Figure 2933: PRO37946

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 Figure 2936: DNA330289, 197444.1, 213567\_at  
 Figure 2937: PRO85516  
 Figure 2938: DNA159560, DNA159560, 213577\_at  
 Figure 2939: DNA330290, 1398807.8, 213581\_at  
 Figure 2940: PRO85517  
 Figure 2941: DNA327799, HSRP26AA, 213587\_s\_at  
 Figure 2942: PRO40011  
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 Figure 2944: PRO61716  
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 Figure 2946: PRO85518  
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 Figure 2950: PRO36437  
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 Figure 2954: PRO61932  
 Figure 2955: DNA270758, HSU54778, 213655\_at  
 Figure 2956: PRO59117  
 Figure 2957: DNA330293, BC011922, 213666\_at  
 Figure 2958: PRO85520  
 Figure 2959: DNA325704, MARS, 213671\_s\_at  
 Figure 2960: PRO82188  
 Figure 2961: DNA304796, NP\_443109.1, 213696\_s\_at  
 Figure 2962: PRO71208  
 Figure 2963: DNA273236, ASAHI, 213702\_x\_at  
 Figure 2964: PRO61263  
 Figure 2965: DNA255913, DNA255913, 213725\_x\_at  
 Figure 2966: DNA328629, TUBB2, 213726\_x\_at  
 Figure 2967: PRO84407  
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Figure 3442: PRO61003  
Figure 3443: DNA327862, NP\_060445.1, 218349\_s\_at  
Figure 3444: PRO83803  
Figure 3445: DNA328854, NP\_056979.1, 218350\_s\_at  
Figure 3446: PRO84585  
Figure 3447A-B: DNA273415, KIF4A, 218355\_at  
Figure 3448: PRO61414  
Figure 3449: DNA324890, NP\_037525.1, 218356\_at  
Figure 3450: PRO81496  
Figure 3451: DNA330365, NP\_036591.1, 218357\_s\_at  
Figure 3452: PRO85580  
Figure 3453A-B: DNA331595, NP\_073602.2, 218376\_s\_at  
Figure 3454: PRO86599  
Figure 3455: DNA330367, NP\_057174.1, 218379\_at  
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Figure 3461A-B: DNA287192, NP\_006178.1, 218400\_at  
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Figure 3463: DNA329912, TTC4, 218442\_at  
Figure 3464: PRO85227

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Figure 3466: PRO12398  
Figure 3467: DNA304781, NP\_057385.2, 218461.at  
Figure 3468: PRO71191  
Figure 3469: DNA328861, NP\_057030.2, 218472.s.at  
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Figure 3473: DNA150648, NP\_037464.1, 218507.at  
Figure 3474: PRO11576  
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Figure 3476: PRO84592  
Figure 3477: DNA330369, NP\_060822.1, 218513.at  
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Figure 3483: DNA330371, NP\_060813.1, 218535.s.at  
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Figure 3499: DNA329286, NP\_005691.2, 218567.x.at  
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Figure 3509: DNA287642, NP\_060934.1, 218597.s.at  
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Figure 3515: DNA327869, NP\_057672.1, 218625.at  
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Figure 3521: DNA330378, HCAP-G, 218663.at  
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Figure 3529: DNA329288, NP\_061910.1, 218695.at  
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Figure 3535: DNA330380, NP\_078937.2, 218722.s.at  
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Figure 3537: DNA324251, NP\_060880.2, 218726.at  
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Figure 3904: DNA272972, NP\_057356.1, 221496\_s\_at  
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Figure 3909: PRO85657  
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Figure 3911: PRO82634  
Figure 3912: DNA328953, NP\_004086.1, 221539\_at  
Figure 3913: PRO70296  
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Figure 3915: PRO81157  
Figure 3916: DNA330457, NP\_076944.1, 221559\_s\_at  
Figure 3917: PRO85658  
Figure 3918: DNA329319, BC006401, 221601\_s\_at  
Figure 3919: PRO1607  
Figure 3920: DNA329319, NP\_005440.1, 221602\_s\_at  
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Figure 3927: PRO85659  
Figure 3928: DNA218280, IL21R, 221658\_s\_at  
Figure 3929: PRO34332  
Figure 3930: DNA327927, NP\_037390.2, 221666\_s\_at  
Figure 3931: PRO57311  
Figure 3932: DNA254777, NP\_055140.1, 221676\_s\_at  
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Figure 3937: PRO85660  
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Figure 3942: DNA328961, MGC17330, 221757\_at  
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Figure 3946: DNA193901, DNA193901, 221768\_at  
Figure 3947: PRO23319  
Figure 3948: DNA328964, AK056028, 221770\_at  
Figure 3949: PRO84669  
Figure 3950: DNA330463, HSM801191, 221790\_s\_at  
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Figure 3953: DNA274058, NP\_057203.1, 221816\_s\_at  
Figure 3954: PRO61999  
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Figure 3956: PRO2733  
Figure 3957: DNA273311, NP\_003022.1, 221833\_at  
Figure 3958: PRO61319  
Figure 3959: DNA272419, AF105036, 221841\_s\_at  
Figure 3960: PRO60672  
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Figure 3964: PRO85664  
Figure 3965A-B: DNA330466, AB018304, 221922\_at  
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Figure 3968: DNA330467, NP\_060114.1, 221986\_s\_at  
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Figure 3970: DNA287235, FLJ10534, 221987\_s\_at  
Figure 3971: PRO69514  
Figure 3972: DNA327114, RPL10, 221989\_at  
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Figure 3981: PRO85234  
Figure 3982: DNA304466, NP\_004834.1, 222062\_at  
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Figure 3985: PRO82139  
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Figure 3988: PRO69490  
Figure 3989: DNA256784, NP\_075069.1, 222209\_s\_at  
Figure 3990: PRO51716  
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Figure 3993: DNA330469, NP\_056249.1, 222250\_s\_at  
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Figure 3995: DNA328885, EKI1, 222262\_s\_at  
Figure 3996: PRO50294  
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Figure 3998: PRO85668  
Figure 3999: DNA330471, 027307.1, 222309\_at  
Figure 4000: PRO85669  
Figure 4001: DNA330472, 128864.1, 222326\_at  
Figure 4002: PRO85670  
Figure 4003: DNA330473, NP\_060676.2, 222387\_s\_at

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 Figure 4042: PRO276  
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 Figure 4055: PRO84914  
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 Figure 4061: PRO83870  
 Figure 4062: DNA327943, NP\_055399.1, 222646\_s\_at  
 Figure 4063: PRO865  
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 Figure 4067: DNA330487, AB052751, 222696\_at  
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 Figure 4074: PRO85683  
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 Figure 4076: PRO85684  
 Figure 4077: DNA330491, BC002522, 222759\_at  
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 Figure 4111: PRO85692

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 Figure 4162A-B: DNA256347, AF298880, 223055\_s\_at  
 Figure 4163: PRO51389  
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 Figure 4378A-B: DNA330555, HSM801768, 224308\_s\_at  
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 Figure 4381: PRO85739  
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Figure 5742: PRO86165  
Figure 5743: DNA331705, 428179.1, 241775\_at  
Figure 5744: PRO86700  
Figure 5745: DNA195721, DNA195721, 241819\_at  
Figure 5746: DNA331009, 222011.1, 241824\_at  
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Figure 5748: DNA331010, 218800.1, 241843\_at  
Figure 5749: PRO86168  
Figure 5750: DNA331011, 979953.1, 241859\_at  
Figure 5751: PRO86169  
Figure 5752: DNA331012, 030070.1, 241869\_at  
Figure 5753: PRO86170  
Figure 5754: DNA331013, 406509.1, 241924\_at  
Figure 5755: PRO86171  
Figure 5756: DNA329506, NP\_387510.1, 241937\_s\_at  
Figure 5757: PRO85067  
Figure 5758: DNA331014, 1447958.2, 241985\_at  
Figure 5759: PRO86172  
Figure 5760: DNA331015, 109159.1, 242031\_at  
Figure 5761: PRO86173  
Figure 5762: DNA331016, 229438.1, 242051\_at  
Figure 5763: PRO86174  
Figure 5764: DNA328213, 419856.5, 242059\_at  
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Figure 5770: DNA331019, 234788.2, 242245\_at  
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Figure 5790: DNA331027, 053796.1, 242560\_at  
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Figure 5792: DNA331028, 7693434.1, 242606\_at  
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Figure 5804A-C: DNA331033, AF330045, 242722\_at  
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Figure 5806: DNA331034, 7689086.1, 242735\_x\_at  
Figure 5807: PRO86192  
Figure 5808: DNA331035, 210512.1, 242783\_at  
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Figure 5816: DNA331709, 017276.1, 242903\_at  
Figure 5817: PRO86704  
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Figure 5827: PRO86201  
Figure 5828: DNA331044, 226264.10, 243154\_at  
Figure 5829: PRO86202  
Figure 5830: DNA331045, 066434.1, 243222\_at  
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Figure 5832: DNA331712, 005752.1, 243271\_at  
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Figure 5835: DNA331048, 7688599.1, 243366.s\_at  
Figure 5836: PRO86206  
Figure 5837: DNA331049, 402027.4, 243395\_at  
Figure 5838: PRO86207  
Figure 5839: DNA331713, 982999.2, 243423\_at  
Figure 5840: PRO86708  
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Figure 5842: PRO86209  
Figure 5843: DNA331714, 332965.1, 243496\_at  
Figure 5844: PRO86709  
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Figure 5846: PRO86211  
Figure 5847: DNA331715, 7683458.1, 243514\_at  
Figure 5848: PRO86710  
Figure 5849: DNA331055, 1512996.3, 243561\_at

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 Figure 5857: PRO86215  
 Figure 5858: DNA331058, 400813.1, 243918\_at  
 Figure 5859: PRO86216  
 Figure 5860: DNA331059, 035870.32, 243934\_at  
 Figure 5861: PRO86217  
 Figure 5862: DNA210271, DNA210271, 243999\_at  
 Figure 5863: PRO33803  
 Figure 5864A-B: DNA331060, 406931.1, 244008\_at  
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 Figure 5866: DNA331061, 198683.4, 244026\_at  
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 Figure 5868: DNA331062, BC021973, 244052\_at  
 Figure 5869: PRO23771  
 Figure 5870: DNA331716, 212607.1, 244267\_at  
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 Figure 5873: PRO86221  
 Figure 5874: DNA108738, DNA108738, 244321\_at  
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 Figure 5885: PRO86225  
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 Figure 5887: PRO85073  
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 Figure 5889: PRO86226  
 Figure 5890: DNA331070, 393412.1, 244801\_at  
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 Figure 5892: DNA331071, 343563.1, 244869\_at  
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 Figure 5897: PRO84667  
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 Figure 5901: DNA327205, GBP5, DNA61875\_at  
 Figure 5902: PRO83478  
 Figure 5903: DNA331717, BC020203, DNA71289\_at
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 Figure 5905: DNA331718, AK024409, DNA92232\_at  
 Figure 5906: PRO86713  
 Figure 5907: DNA96866, DNA96866, DNA96866\_at  
 Figure 5908: PRO6015  
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 Figure 5911: DNA108670, DNA108670,  
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 Figure 5912: PRO7171  
 Figure 5913: DNA304467, BC004535, DNA108688\_at  
 Figure 5914: PRO71043  
 Figure 5915A-B: DNA108728, DNA108728,  
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 Figure 5916: PRO9741  
 Figure 5917: DNA329215, ICOS, DNA108917\_at  
 Figure 5918: PRO7424  
 Figure 5919: DNA331719, BC002424, DNA143288\_at  
 Figure 5920: PRO12705  
 Figure 5921A-B: DNA150956, HUMORFKG1P,  
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 Figure 5922: DNA330417, APOL6, DNA164989\_at  
 Figure 5923: PRO21341  
 Figure 5924: DNA329483, AF384857, DNA166819\_at  
 Figure 5925: PRO20110  
 Figure 5926: DNA26842, DNA26842, P\_Z64949\_at  
 Figure 5927: PRO180  
 Figure 5928: DNA304468, NP\_077300.1, P\_Z93700\_at  
 Figure 5929: PRO71044  
 Figure 5930: DNA39423, DNA39423, P\_X52252\_at  
 Figure 5931: PRO271  
 Figure 5932: DNA330262, GW112, P\_Z64962\_at  
 Figure 5933: PRO85493  
 Figure 5934: DNA331074, AF252257, P\_A37030\_at  
 Figure 5935: DNA60764, DNA60764, P\_A46906\_at  
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 Figure 5937: DNA331720, AF289594, P\_A37063\_at  
 Figure 5938: PRO86714  
 Figure 5939: DNA331721, BC017876, P\_A37079\_at  
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 Figure 5942: PRO1575  
 Figure 5943: DNA304475, NP\_116246.1, P\_A37128\_at  
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 Figure 5946: PRO1207  
 Figure 5947: DNA88195, CD3G, NM\_000073\_at  
 Figure 5948: PRO2693  
 Figure 5949: DNA325712, CDK4, NM\_000075\_at  
 Figure 5950: PRO82194  
 Figure 5951: DNA329934, BC013083, NM\_000099\_at  
 Figure 5952: PRO2721  
 Figure 5953A-B: DNA331722, HUMFVA,  
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 Figure 5954: PRO36374  
 Figure 5955: DNA331723, U66095, NM\_000161\_at

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 Figure 5957: DNA227668, HUMGLYKINB,  
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 Figure 5958: PRO38131  
 Figure 5959A-D: DNA331724, HSGLA,  
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 Figure 5960: DNA331725, BC006342, NM\_000175\_at  
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 Figure 5963: DNA331726, HUMICAMA1A,  
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 Figure 5964: PRO86716  
 Figure 5965A-B: DNA88419, HSINTA6R,  
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 Figure 5966: PRO2339  
 Figure 5967: DNA88428, HUMLAP, NM\_000211\_at  
 Figure 5968: PRO2787  
 Figure 5969: DNA226014, NP\_000230.1,  
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 Figure 5971: DNA97287, NP\_000240.1,  
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 Figure 5972: PRO3634  
 Figure 5973: DNA88554, NP\_000241.1,  
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 Figure 5974: PRO2839  
 Figure 5975: DNA331727, BC008015, NM\_000269\_at  
 Figure 5976: PRO37534  
 Figure 5977A-E: DNA331728, PTEN4, NM\_000314\_at  
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 Figure 5981: PRO37544  
 Figure 5982: DNA76512, HSIL2REC, NM\_000417\_at  
 Figure 5983: PRO2020  
 Figure 5984: DNA76514, HSIL4R, NM\_000418\_at  
 Figure 5985: PRO2540  
 Figure 5986: DNA329522, NP\_000433.2,  
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 Figure 5987: PRO85080  
 Figure 5988: DNA188732, NP\_000475.1,  
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 Figure 5996: DNA36718, HUMIL10, NM\_000572\_at  
 Figure 5997: PRO73  
 Figure 5998: DNA324158, NP\_000567.1,  
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- Figure 5999: PRO65  
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 Figure 6003: PRO70536  
 Figure 6004: DNA216500, NP\_000575.1,  
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 Figure 6005: PRO34252  
 Figure 6006: DNA36712, HUMIL3, NM\_000588\_at  
 Figure 6007: PRO67  
 Figure 6008A-B: DNA331733, AF361105,  
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 Figure 6009: DNA331734, BC014081, NM\_000593\_at  
 Figure 6010: PRO36996  
 Figure 6011A-B: DNA331735, AY066019,  
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 Figure 6012A-B: DNA331736, AY070490,  
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 Figure 6013: DNA331737, BC009902, NM\_000597\_at  
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 Figure 6015: DNA217246, NP\_000591.1,  
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 Figure 6016: PRO34288  
 Figure 6017: DNA331075, NP\_000601.2,  
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 Figure 6018: PRO86231  
 Figure 6019A-C: DNA331738, AF375790,  
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 Figure 6020A-B: DNA220752, ITGAM,  
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 Figure 6021: PRO34730  
 Figure 6022A-B: DNA97288, HUMBCL2C,  
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 Figure 6023: PRO3635  
 Figure 6024: DNA331739, A12178, NM\_000636\_at  
 Figure 6025: PRO86720  
 Figure 6026: DNA331740, HUMHPC, NM\_000639\_at  
 Figure 6027: PRO1208  
 Figure 6028: DNA329000, HSU03905, NM\_000647\_at  
 Figure 6029: PRO84690  
 Figure 6030: DNA328253, NP\_004029.1,  
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 Figure 6031: PRO84149  
 Figure 6032: DNA89242, ANXA1, NM\_000700\_at  
 Figure 6033: PRO2907  
 Figure 6034: DNA88194, CD3E, NM\_000733\_at  
 Figure 6035: PRO2220  
 Figure 6036: DNA329975, PRO2325, NM\_000791\_at  
 Figure 6037: DNA331741, BC003097, NM\_000873\_at  
 Figure 6038: PRO86721  
 Figure 6039: DNA331076, HSIFNABR,  
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 Figure 6040: PRO86232  
 Figure 6041A-B: DNA83101, NP\_000868.1,

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Figure 6042: PRO2590  
Figure 6043A-B: DNA76508, A07795, NM\_000878\_at  
Figure 6044: PRO2538  
Figure 6045: DNA36714, NP\_000870.1,  
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Figure 6046: PRO69  
Figure 6047A-B: DNA88417, HSINTAL4,  
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Figure 6048: PRO2337  
Figure 6049: DNA88433, HUMINTB7A,  
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Figure 6050: PRO2346  
Figure 6051: DNA226053, NP\_000908.1,  
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Figure 6052: PRO36516  
Figure 6053A-B: DNA331742, BC018127,  
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Figure 6054: PRO86722  
Figure 6055: DNA227709, PTGER2, NM\_000956\_at  
Figure 6056: PRO38172  
Figure 6057: DNA226195, NP\_000949.1,  
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Figure 6058: PRO36658  
Figure 6059: DNA327639, TCN1, NM\_001062\_at  
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Figure 6062: PRO12446  
Figure 6063: DNA171404, HSU45878, NM\_001165\_at  
Figure 6064: PRO20132  
Figure 6065: DNA331743, AAA19687.1,  
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Figure 6066: PRO12242  
Figure 6067A-B: DNA325972, BC018739,  
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Figure 6068: PRO82417  
Figure 6069: DNA287267, CCNA2, NM\_001237\_at  
Figure 6070: PRO37015  
Figure 6071: DNA196674, D86042, NM\_001243\_at  
Figure 6072: PRO2938  
Figure 6073: DNA325568, BC017575, NM\_001274\_at  
Figure 6074: PRO12187  
Figure 6075: DNA226177, CCR1, NM\_001295\_at  
Figure 6076: PRO36640  
Figure 6077: DNA331744, CTSW, NM\_001335\_at  
Figure 6078: PRO1574  
Figure 6079: DNA93466, HUMEDG, NM\_001400\_at  
Figure 6080: PRO4936  
Figure 6081: DNA331745, HSU77085, NM\_001423\_at  
Figure 6082: PRO12467  
Figure 6083A-C: DNA151167, HSABP280,  
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Figure 6084: PRO12867  
Figure 6085A-C: DNA331746, AF043045,  
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Figure 6086: PRO86723  
Figure 6087: DNA188346, FLT3LG, NM\_001459\_at  
Figure 6088: PRO21766  
Figure 6089: DNA227173, HSU93049, NM\_001465\_at  
Figure 6090: PRO37636  
Figure 6091A-B: DNA331747, GABBR1,  
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Figure 6092: PRO86724  
Figure 6093A-B: DNA76503, IL10RA, NM\_001558\_at  
Figure 6094: PRO2536  
Figure 6095A-B: DNA227750, IL12RB2,  
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Figure 6096: PRO38213  
Figure 6097: DNA76556, HSU03397, NM\_001561\_at  
Figure 6098: PRO2023  
Figure 6099: DNA82362, CXCL10, NM\_001565\_at  
Figure 6100: PRO1718  
Figure 6101: DNA227013, NP\_001560.1,  
NM\_001569\_at  
Figure 6102: PRO37476  
Figure 6103: DNA331748, BC009799, NM\_001657\_at  
Figure 6104: PRO46  
Figure 6105: DNA150716, HSZNFNPRA,  
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Figure 6106: PRO12790  
Figure 6107: DNA331077, HUMBGPAB,  
NM\_001712\_at  
Figure 6108: PRO86233  
Figure 6109: DNA150718, NP\_001727.1,  
NM\_001736\_at  
Figure 6110: PRO12435  
Figure 6111A-B: DNA226387, HSCYCLF,  
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Figure 6112: PRO36850  
Figure 6113: DNA329002, CCT6A, NM\_001762\_at  
Figure 6114: PRO4912  
Figure 6115: DNA226380, HSCD37, NM\_001774\_at  
Figure 6116: PRO4695  
Figure 6117: DNA331749, D84277, NM\_001775\_at  
Figure 6118: PRO86725  
Figure 6119: DNA88199, HUMMEMGL1,  
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Figure 6120: PRO2696  
Figure 6121: DNA226436, CD69, NM\_001781\_at  
Figure 6122: PRO36899  
Figure 6123: DNA331750, A23013, NM\_001803\_at  
Figure 6124: PRO2496  
Figure 6125: DNA151798, NP\_001797.1,  
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Figure 6126: PRO12186  
Figure 6127: DNA227232, SLC31A1, NM\_001859\_at  
Figure 6128: PRO37695  
Figure 6129: DNA331751, S68134, NM\_001881\_at  
Figure 6130: PRO86726  
Figure 6131: DNA331078, NP\_001894.1,  
NM\_001903\_at

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 Figure 6134: PRO86727  
 Figure 6135: DNA225810, HSCATHL, NM\_001912\_at  
 Figure 6136: PRO36273  
 Figure 6137: DNA83048, DEFA4, NM\_001925\_at  
 Figure 6138: PRO2057  
 Figure 6139: DNA88215, NP\_001919.1, NM\_001928\_at  
 Figure 6140: PRO2703  
 Figure 6141: DNA196562, HSPCHDP7, NM\_001935\_at  
 Figure 6142: PRO25042  
 Figure 6143: DNA226871, NP\_001942.1, NM\_001951\_at  
 Figure 6144: PRO37334  
 Figure 6145: DNA227332, NP\_001943.1, NM\_001952\_at  
 Figure 6146: PRO37795  
 Figure 6147: DNA225661, ECGF1, NM\_001953\_at  
 Figure 6148: PRO36124  
 Figure 6149: DNA273174, HSEF1DELA, NM\_001960\_at  
 Figure 6150: PRO61211  
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 Figure 6152: PRO12798  
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 Figure 6154: PRO38010  
 Figure 6155: DNA331754, BC002464, NM\_001992\_at  
 Figure 6156: PRO86728  
 Figure 6157: DNA331755, D83920, NM\_002003\_at  
 Figure 6158: PRO86729  
 Figure 6159: DNA226881, HUMERGBFLI, NM\_002017\_at  
 Figure 6160: PRO37344  
 Figure 6161: DNA88332, FVT1, NM\_002035\_at  
 Figure 6162: PRO2753  
 Figure 6163: DNA225979, G1P3, NM\_002038\_at  
 Figure 6164: PRO36442  
 Figure 6165: DNA331756, BC002666, NM\_002053\_at  
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 Figure 6167: DNA88374, GZMK, NM\_002104\_at  
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 Figure 6169: DNA228014, ICAM3, NM\_002162\_at  
 Figure 6170: PRO38477  
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 Figure 6173: DNA76517, IL7R, NM\_002185\_at  
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 Figure 6175: DNA188271, NP\_002179.1, NM\_002188\_at  
 Figure 6176: PRO21795  
 Figure 6177: DNA226396, IL15RA, NM\_002189\_at  
 Figure 6178: PRO36859
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 Figure 6180: PRO37477  
 Figure 6181A-B: DNA88427, HSFNRB, NM\_002211\_at  
 Figure 6182: PRO2786  
 Figure 6183: DNA103215, NP\_002210.1, NM\_002219\_at  
 Figure 6184: PRO4545  
 Figure 6185: DNA331758, S82269, NM\_002222\_at  
 Figure 6186: PRO86731  
 Figure 6187: DNA331759, BC002646, NM\_002228\_at  
 Figure 6188: PRO4671  
 Figure 6189: DNA331760, BC009466, NM\_002229\_at  
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 Figure 6191A-B: DNA331761, AF305731S5, NM\_002250\_at  
 Figure 6192: DNA150971, KLRB1, NM\_002258\_at  
 Figure 6193: PRO12564  
 Figure 6194: DNA326343, BC003572, NM\_002265\_at  
 Figure 6195: PRO82739  
 Figure 6196: DNA288243, LAG3, NM\_002286\_at  
 Figure 6197: PRO36451  
 Figure 6198A-B: DNA188301, LIF, NM\_002309\_at  
 Figure 6199: PRO21834  
 Figure 6200A-B: DNA331762, HUMLYTOXBB, NM\_002341\_at  
 Figure 6201: DNA88666, NP\_002334.1, NM\_002343\_at  
 Figure 6202: PRO2892  
 Figure 6203: DNA227150, LY6E, NM\_002346\_at  
 Figure 6204: PRO37613  
 Figure 6205: DNA327255, BC001061, NM\_002394\_at  
 Figure 6206: PRO57298  
 Figure 6207: DNA150937, HSU94352, NM\_002405\_at  
 Figure 6208: PRO11598  
 Figure 6209: DNA82376, CXCL9, NM\_002416\_at  
 Figure 6210: PRO1723  
 Figure 6211: DNA103283, MNDA, NM\_002432\_at  
 Figure 6212: PRO4613  
 Figure 6213: DNA103525, NP\_002457.1, NM\_002466\_at  
 Figure 6214: PRO4852  
 Figure 6215A-B: DNA331763, AF058696, NM\_002485\_at  
 Figure 6216: PRO36001  
 Figure 6217: DNA103382, HSU49395, NM\_002561\_at  
 Figure 6218: PRO4711  
 Figure 6219A-B: DNA88331, HSFUR, NM\_002569\_at  
 Figure 6220: PRO2752  
 Figure 6221: DNA103488, PCNA, NM\_002592\_at  
 Figure 6222: PRO4815  
 Figure 6223: DNA328587, NP\_002612.1, NM\_002621\_at  
 Figure 6224: PRO2854  
 Figure 6225: DNA331764, NP\_071438.1,

NM\_002624\_at  
 Figure 6226: PRO86732  
 Figure 6227: DNA227067, HSPKCB1A,  
 NM\_002738\_at  
 Figure 6228: PRO37530  
 Figure 6229: DNA227090, NP\_002750.1,  
 NM\_002759\_at  
 Figure 6230: PRO37553  
 Figure 6231: DNA88626, HUMSAPABCD,  
 NM\_002778\_at  
 Figure 6232: PRO2875  
 Figure 6233: DNA329098, BC007897, NM\_002808\_at  
 Figure 6234: PRO84749  
 Figure 6235: DNA326853, NP\_002818.1,  
 NM\_002827\_at  
 Figure 6236: PRO38066  
 Figure 6237: DNA88607, NP\_002892.1,  
 NM\_002901\_at  
 Figure 6238: PRO2863  
 Figure 6239: DNA331765, AF294009, NM\_002934\_at  
 Figure 6240: PRO2444  
 Figure 6241: DNA331766, AF043339, NM\_002983\_at  
 Figure 6242: DNA51778, HSHC21, NM\_002984\_at  
 Figure 6243: PRO80  
 Figure 6244: DNA330124, CCL22, NM\_002990\_at  
 Figure 6245: PRO34107  
 Figure 6246: DNA227788, NP\_002995.1,  
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 Figure 6247: PRO38251  
 Figure 6248: DNA329005, HSU76367, NM\_003037\_at  
 Figure 6249: PRO12612  
 Figure 6250: DNA196489, HUMMCT, NM\_003051\_at  
 Figure 6251: PRO24980  
 Figure 6252A-B: DNA103542, HSLR11,  
 NM\_003105\_at  
 Figure 6253: PRO4869  
 Figure 6254: DNA331767, D78130, NM\_003129\_at  
 Figure 6255: PRO37946  
 Figure 6256: DNA328259, AF029311, NM\_003150\_at  
 Figure 6257: DNA227447, HSTCF1C, NM\_003202\_at  
 Figure 6258: PRO37910  
 Figure 6259A-B: DNA226536, HSTRR,  
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 Figure 6260: PRO36999  
 Figure 6261A-B: DNA83176, TGFB3,  
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 Figure 6262: PRO2620  
 Figure 6263: DNA103532, TM7SF1, NM\_003272\_at  
 Figure 6264: PRO4859  
 Figure 6265A-B: DNA103585, HUMTOPI,  
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 Figure 6266: PRO4909  
 Figure 6267: DNA331768, BC007935, NM\_003316\_at  
 Figure 6268: PRO22907  
 Figure 6269: DNA331769, AF065241, NM\_003329\_at  
 Figure 6270A-B: DNA331770, AF019563,

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 Figure 6271: DNA331771, HSU76367, NM\_003355\_at  
 Figure 6272: PRO86733  
 Figure 6273: DNA151906, HSUNG, NM\_003362\_f\_at  
 Figure 6274: PRO12214  
 Figure 6275: DNA103380, HUMVDAC1X,  
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 Figure 6276: PRO4710  
 Figure 6277: DNA225992, NP\_003374.1,  
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 Figure 6278: PRO36455  
 Figure 6279: DNA227330, NP\_003443.1,  
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 Figure 6280: PRO37793  
 Figure 6281: DNA93449, AF025375, NM\_003467\_at  
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 Figure 6283: DNA331772, BC010022, NM\_003504\_at  
 Figure 6284: PRO71058  
 Figure 6285: DNA331773, AF123318, NM\_003550\_at  
 Figure 6286: PRO86734  
 Figure 6287: DNA331079, AF036342, NM\_003650\_at  
 Figure 6288: PRO1191  
 Figure 6289: DNA328260, AF305428, NM\_003661\_at  
 Figure 6290: PRO84152  
 Figure 6291: DNA151802, AB004066, NM\_003670\_at  
 Figure 6292: PRO12890  
 Figure 6293: DNA227213, NP\_003671.1,  
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 Figure 6294: PRO37676  
 Figure 6295: DNA331774, AK001769, NM\_003730\_at  
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 Figure 6297: DNA150433, AB005043, NM\_003745\_at  
 Figure 6298: PRO12771  
 Figure 6299: DNA328377, NP\_003759.1,  
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 Figure 6300: PRO84232  
 Figure 6301: DNA194746, HSM800355,  
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 Figure 6302: DNA196431, AF064090, NM\_003807\_at  
 Figure 6303: PRO5810  
 Figure 6304: DNA61870, HSU57059, NM\_003810\_at  
 Figure 6305: PRO1096  
 Figure 6306A-B: DNA200236, NP\_003807.1,  
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 Figure 6307: PRO34137  
 Figure 6308: DNA331775, BC000334, NM\_003824\_at  
 Figure 6309: PRO4801  
 Figure 6310: DNA331080, NP\_003835.2,  
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 Figure 6311: PRO86235  
 Figure 6312: DNA225550, IL18RAP, NM\_003853\_at  
 Figure 6313: PRO36013  
 Figure 6314: DNA103451, IL18R1, NM\_003855\_at  
 Figure 6315: PRO4778  
 Figure 6316: DNA151011, AF037989, NM\_003877\_at  
 Figure 6317: PRO12839

Figure 6318: DNA331776, IER3, NM\_003897\_at  
 Figure 6319: PRO84760  
 Figure 6320A-B: DNA150765, SLC7A6, NM\_003983\_at  
 Figure 6321: PRO12458  
 Figure 6322: DNA88308, HUMFCREA, NM\_004106\_at  
 Figure 6323: PRO2739  
 Figure 6324A-B: DNA331777, AF200219S2, NM\_004107\_at  
 Figure 6325: DNA227133, GBP2, NM\_004120\_at  
 Figure 6326: PRO37596  
 Figure 6327: DNA83091, HUMSP13E, NM\_004131\_at  
 Figure 6328: PRO2081  
 Figure 6329A-B: DNA151108, SREBF1, NM\_004176\_at  
 Figure 6330: PRO12105  
 Figure 6331: DNA218676, AF125304, NM\_004195\_at  
 Figure 6332: PRO34454  
 Figure 6333: DNA103394, HSU81800, NM\_004207\_at  
 Figure 6334: PRO4722  
 Figure 6335: DNA329533, BC018782, NM\_004221\_at  
 Figure 6336: PRO85085  
 Figure 6337: DNA331778, AK027513, NM\_004265\_at  
 Figure 6338: PRO86736  
 Figure 6339: DNA151142, NP\_004321.1, NM\_004330\_at  
 Figure 6340: PRO12110  
 Figure 6341: DNA227303, NP\_004322.1, NM\_004331\_at  
 Figure 6342: PRO37766  
 Figure 6343: DNA287240, BST2, NM\_004335\_at  
 Figure 6344: PRO29371  
 Figure 6345: DNA225910, NP\_004336.1, NM\_004345\_at  
 Figure 6346: PRO36373  
 Figure 6347: DNA331779, CASP3, NM\_004346\_at  
 Figure 6348: PRO12832  
 Figure 6349A-B: DNA326191, NP\_004351.1, NM\_004360\_at  
 Figure 6350: PRO2672  
 Figure 6351A-C: DNA150729, HSU47741, NM\_004380\_at  
 Figure 6352: PRO12147  
 Figure 6353A-B: DNA151420, S40832, NM\_004430\_at  
 Figure 6354: PRO12876  
 Figure 6355A-B: DNA218283, EPHB6, NM\_004445\_at  
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 Figure 6357: DNA331780, BC003110, NM\_004512\_at  
 Figure 6358: PRO4843  
 Figure 6359: DNA150935, NP\_004547.1, NM\_004556\_at  
 Figure 6360: PRO12155  
 Figure 6361A-B: DNA151831, NP\_004564.1,  
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 Figure 6362: PRO12198  
 Figure 6363: DNA328262, HSU57094, NM\_004580\_at  
 Figure 6364: PRO84153  
 Figure 6365: DNA331781, HSU77035, NM\_004591\_at  
 Figure 6366: PRO1724  
 Figure 6367: DNA331782, HUMVAIPR, NM\_004624\_at  
 Figure 6368: DNA329984, WRB, NM\_004627\_at  
 Figure 6369: PRO11656  
 Figure 6370: DNA329119, NP\_004633.1, NM\_004642\_at  
 Figure 6371: PRO4550  
 Figure 6372: DNA328578, NP\_004656.2, NM\_004665\_at  
 Figure 6373: PRO7426  
 Figure 6374: DNA331783, BC011726, NM\_004706\_at  
 Figure 6375: PRO86737  
 Figure 6376: DNA218284, AF053004, NM\_004843\_at  
 Figure 6377: PRO34336  
 Figure 6378: DNA151017, AB005047, NM\_004844\_at  
 Figure 6379: PRO12841  
 Figure 6380A-B: DNA150447, AB011098, NM\_004863\_at  
 Figure 6381: PRO12256  
 Figure 6382: DNA88295, HUMERP72H, NM\_004911\_at  
 Figure 6383: PRO2733  
 Figure 6384: DNA331784, AB001325, NM\_004925\_at  
 Figure 6385: PRO380281  
 Figure 6386A-B: DNA331785, DSC1, NM\_004948\_at  
 Figure 6387: PRO36355  
 Figure 6388: DNA227563, NP\_004946.1, NM\_004955\_at  
 Figure 6389: PRO38026  
 Figure 6390: DNA331786, HUMSTPK13, NM\_005030\_at  
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 Figure 6392: DNA329011, BCL3, NM\_005178\_at  
 Figure 6393: PRO4785  
 Figure 6394: DNA331787, AF213050, NM\_005192\_at  
 Figure 6395: PRO86739  
 Figure 6396: DNA103330, HUMPOPSTK, NM\_005204\_at  
 Figure 6397: PRO4660  
 Figure 6398: DNA331788, HUMIGCTL3, NM\_005214\_at  
 Figure 6399: DNA325060, NP\_004075.1, NM\_005217\_at  
 Figure 6400: PRO2570  
 Figure 6401: DNA331789, HSCFOS, NM\_005252\_at  
 Figure 6402: DNA304668, HSPA1A, NM\_005346\_at  
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 Figure 6404: DNA331790, HUMCMYBA, NM\_005375\_at  
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 Figure 6408: PRO19838  
 Figure 6409: DNA329319, TOSO, NM\_005449\_at  
 Figure 6410: PRO1607  
 Figure 6411A-B: DNA189702, AF047348,  
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 Figure 6412: PRO22775  
 Figure 6413: DNA150989, HSP27, NM\_005532\_at  
 Figure 6414: PRO12569  
 Figure 6415A-C: DNA331792, HUMOP18A,  
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 Figure 6416: DNA97285, LDHA, NM\_005566\_at  
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 Figure 6418: DNA225675, LMAN1, NM\_005570\_at  
 Figure 6419: PRO36138  
 Figure 6420: DNA331793, AF148645, NM\_005614\_at  
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 Figure 6422: DNA331794, BC001263, NM\_005627\_at  
 Figure 6423: PRO86741  
 Figure 6424: DNA226500, NP\_005619.1,  
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 Figure 6425: PRO36963  
 Figure 6426A-B: DNA227206, NP\_005648.1,  
 NM\_005657\_at  
 Figure 6427: PRO37669  
 Figure 6428: DNA323937, NP\_005689.2,  
 NM\_005698\_at  
 Figure 6429: PRO80670  
 Figure 6430: DNA331081, NP\_005714.2,  
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 Figure 6431: PRO4845  
 Figure 6432: DNA304459, PPIF, NM\_005729\_at  
 Figure 6433: PRO37073  
 Figure 6434A-B: DNA331082, AF057299,  
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 Figure 6435: PRO86236  
 Figure 6436: DNA88541, PBEF, NM\_005746\_at  
 Figure 6437: PRO2834  
 Figure 6438: DNA329014, EBI3, NM\_005755\_at  
 Figure 6439: PRO9998  
 Figure 6440: DNA93548, NP\_005758.1,  
 NM\_005767\_at  
 Figure 6441: PRO4929  
 Figure 6442: DNA331083, NP\_005759.2,  
 NM\_005768\_at  
 Figure 6443: PRO86237  
 Figure 6444: DNA193866, AF081675, NM\_005810\_at  
 Figure 6445: PRO23288  
 Figure 6446: DNA75525, GPA33, NM\_005814\_at  
 Figure 6447: PRO2524  
 Figure 6448A-B: DNA88650, TACTILE,  
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 Figure 6449: PRO2460  
 Figure 6450: DNA150959, NP\_005813.1,

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 Figure 6452: DNA329538, BC001731, NM\_005898\_at  
 Figure 6453: PRO85088  
 Figure 6454: DNA324110, MDH1, NM\_005917\_at  
 Figure 6455: PRO4918  
 Figure 6456: DNA328266, NP\_005993.1,  
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 Figure 6457: PRO12125  
 Figure 6458: DNA150941, NP\_006012.1,  
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 Figure 6459: PRO12548  
 Figure 6460: DNA227138, NP\_006045.1,  
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 Figure 6461: PRO37601  
 Figure 6462: DNA88614, HSRING6, NM\_006120\_at  
 Figure 6463: PRO2867  
 Figure 6464: DNA331795, NP\_006129.2,  
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 Figure 6465: PRO81984  
 Figure 6466: DNA331796, HUMCD284,  
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 Figure 6467: DNA330114, GPR19, NM\_006143\_at  
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 Figure 6469: DNA88372, HUMHFSP, NM\_006144\_at  
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 Figure 6471: DNA103526, HSU10485, NM\_006152\_at  
 Figure 6472: PRO4853  
 Figure 6473: DNA331797, BC020544, NM\_006159\_at  
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 Figure 6477A-B: DNA151841, HUMA20,  
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 Figure 6479: DNA331798, TSG101, NM\_006292\_at  
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 Figure 6481: DNA83109, HUMIIP, NM\_006332\_at  
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 Figure 6489: DNA331801, BC012589, NM\_006419\_at  
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 Figure 6497: DNA151804, RELB, NM\_006509\_at  
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 Figure 6517: DNA227035, HUMHUMCM5,  
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 Figure 6535: DNA328271, ZWINT, NM\_007057\_at  
 Figure 6536: PRO81868  
 Figure 6537: DNA331809, NP\_009046.1,  
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 Figure 6539: DNA103587, HSMRL3R, NM\_007208\_at  
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 Figure 6541: DNA330180, TRC8, NM\_007218\_at  
 Figure 6542: PRO85428  
 Figure 6543: DNA331810, HSU64805, NM\_007295\_at

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 Figure 6554: PRO1471  
 Figure 6555A-B: DNA227255, STAG3,  
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 Figure 6559: DNA88510, HSNKG5, NM\_012483\_at  
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 Figure 6567: DNA103481, HUMAUANTIG,  
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 Figure 6568: PRO4808  
 Figure 6569: DNA196426, H963, NM\_013308\_at  
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 Figure 6574: PRO11576  
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 Figure 6577: DNA304461, HSPC067, NM\_014158\_at  
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 Figure 6579: DNA331814, BC009642, NM\_014164\_at  
 Figure 6580: PRO86751  
 Figure 6581: DNA330374, ORMDL2, NM\_014182\_at  
 Figure 6582: PRO23321  
 Figure 6583: DNA88203, CD5, NM\_014207\_at  
 Figure 6584: PRO2698  
 Figure 6585A-B: DNA331815, AF135372,  
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 Figure 6586: DNA331816, BC003067, NM\_014330\_at  
 Figure 6587: PRO12543  
 Figure 6588: DNA331817, NP\_055154.2,  
 NM\_014339\_at

- Figure 6589: PRO86240  
 Figure 6590: DNA227233, NP\_055157.1,  
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 Figure 6592: DNA227351, AF191020, NM\_014367\_at  
 Figure 6593: PRO37814  
 Figure 6594: DNA331088, NP\_055252.2,  
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 Figure 6595: PRO80674  
 Figure 6596: DNA330084, SIT, NM\_014450\_at  
 Figure 6597: PRO9895  
 Figure 6598: DNA324198, NP\_055400.1,  
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 Figure 6599: PRO37675  
 Figure 6600A-B: DNA151879, NP\_055463.1,  
 NM\_014648\_at  
 Figure 6601: PRO12743  
 Figure 6602: DNA194805, NP\_055500.1,  
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 Figure 6608A-B: DNA277809, KIAA0275,  
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 Figure 6614A-B: DNA150954, KIAA0022,  
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 Figure 6616A-B: DNA227293, DNA227293,  
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 Figure 6617: PRO37756  
 Figure 6618: DNA150805, FAM3C, NM\_014888\_at  
 Figure 6619: PRO11583  
 Figure 6620A-B: DNA194837, NP\_055714.1,  
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 Figure 6622A-B: DNA304464, CHSY1,  
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 Figure 6623: PRO71042  
 Figure 6624: DNA330103, MD-2, NM\_015364\_at  
 Figure 6625: PRO19671  
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 Figure 6627: PRO12814  
 Figure 6628: DNA328590, BC001232, NM\_015864\_at  
 Figure 6629: PRO84375
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 Figure 6631: PRO19859  
 Figure 6632: DNA150865, LOC51596, NM\_015921\_at  
 Figure 6633: PRO11587  
 Figure 6634: DNA150832, NP\_057019.2,  
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 Figure 6646: DNA329292, AF085360, NM\_016101\_at  
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 Figure 6652: DNA328831, AF126780, NM\_016245\_at  
 Figure 6653: PRO233  
 Figure 6654: DNA328513, AF151895, NM\_016283\_at  
 Figure 6655: PRO37815  
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 Figure 6657: PRO71191  
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 Figure 6659: PRO1080  
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 Figure 6662: DNA330390, AF178985, NM\_016546\_at  
 Figure 6663: PRO85599  
 Figure 6664: DNA331822, AF318357, NM\_016553\_at  
 Figure 6665: PRO86753  
 Figure 6666: DNA227298, NP\_057649.1,  
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 Figure 6667: PRO37761  
 Figure 6668: DNA327869, NRN1, NM\_016588\_at  
 Figure 6669: PRO1898  
 Figure 6670: DNA331823, AK027682, NM\_017424\_at  
 Figure 6671: PRO86754  
 Figure 6672: DNA225694, FLJ20005, NM\_017617\_at  
 Figure 6673: PRO36157  
 Figure 6674: DNA326385, NP\_060117.2,  
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 Figure 6675: PRO82778  
 Figure 6676: DNA287206, FLJ20073, NM\_017654\_at  
 Figure 6677: PRO69488  
 Figure 6678: DNA227294, FLJ20303, NM\_017755\_at  
 Figure 6679: PRO37757

Figure 6680: DNA226646, NP\_060352.1,  
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Figure 6681: PRO37109

Figure 6682: DNA331824, BC010907, NM\_017906\_at

Figure 6683: PRO86755

Figure 6684: DNA330537, HELLS, NM\_018063\_at

Figure 6685: PRO81892

Figure 6686: DNA328628, BC011983, NM\_018072\_at

Figure 6687: PRO84406

Figure 6688: DNA328841, BC003082, NM\_018087\_at

Figure 6689: PRO84575

Figure 6730: DNA327199, DJ971N18.2,  
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Figure 6731: PRO83475

Figure 6732: DNA227276, NP\_005702.1,  
NM\_021618\_at

Figure 6733: PRO37739

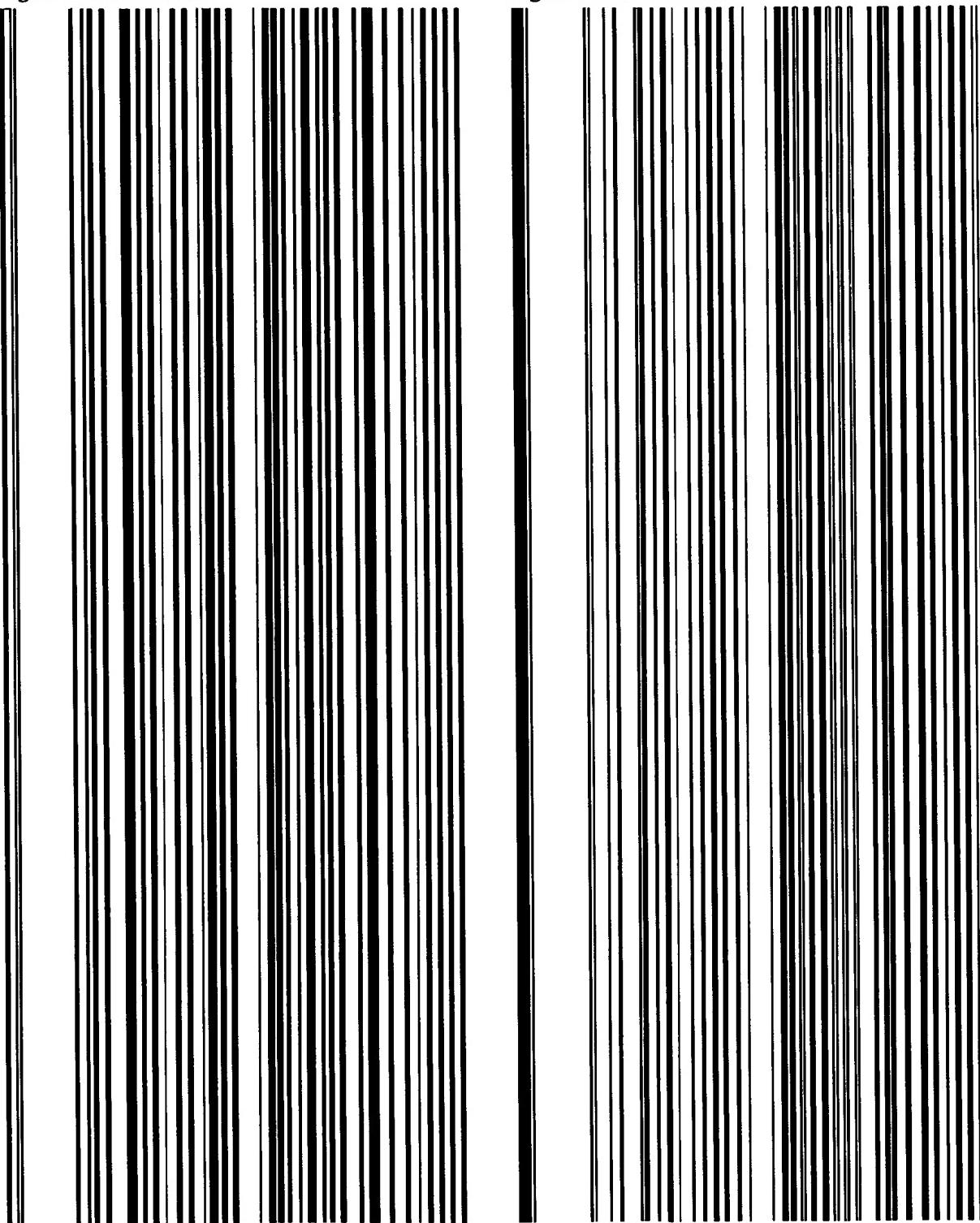
Figure 6734A-B: DNA331832, AF051850,

NM\_021738\_at

Figure 6735: PRO86758

Figure 6736: DNA331833, AF269133, NM\_021798\_at

Figure 6737: PRO86759



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HUMKG1BB\_at  
 Figure 6776: PRO86762  
 Figure 6777A-B: DNA331842, BC004375, AF261758\_at  
 Figure 6778: PRO38492  
 Figure 6779: DNA331095, NP\_005216.1, HUME2F\_at  
 Figure 6780: PRO86245  
 Figure 6781: DNA331843, AF202723, AB014568\_at  
 Figure 6782: DNA159542, DNA159542, HUMMAC30X\_at  
 Figure 6783: DNA331844, BC009267, HUMLAMBBA\_at  
 Figure 6784: PRO82888  
 Figure 6785: DNA331096, S75881, P\_V84330\_at  
 Figure 6786: PRO86246  
 Figure 6787: DNA287239, AF212242, AK024843\_at  
 Figure 6788: PRO38497  
 Figure 6789: DNA154390, DNA154390, HUMP13KIN\_at  
 Figure 6790: DNA151247, DNA151247, P\_V43601\_at  
 Figure 6791: PRO11643  
 Figure 6792: DNA329950, MGC5576, P\_V43613\_at  
 Figure 6793: PRO11558  
 Figure 6794: DNA161927, DNA161927, P\_Z29229\_at  
 Figure 6795: DNA155316, DNA155316, P\_A09058\_at  
 Figure 6796: DNA329026, AF230200, AK021966\_at  
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 Figure 6798: PRO38515  
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 Figure 6800: DNA331845, AK027432, HSM800284\_at  
 Figure 6801: PRO86763  
 Figure 6802: DNA329430, SPPL2A, AX027882\_at  
 Figure 6803: PRO38524  
 Figure 6804: DNA151422, DNA151422, P\_X04312\_at  
 Figure 6805: PRO11792  
 Figure 6806: DNA228066, NP\_079431.1, AK021910\_at  
 Figure 6807: PRO38529  
 Figure 6808A-C: DNA330360, FYCO1, AK023397\_at  
 Figure 6809: PRO85576  
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 Figure 6811: PRO37492  
 Figure 6812: DNA331846, AF272741, HUMTCBYY\_at  
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 Figure 6834: PRO23400  
 Figure 6835: DNA194019, DNA194019, AK000004\_at  
 Figure 6836: PRO23421  
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 Figure 6838: PRO23460  
 Figure 6839: DNA83046, NP\_000565.1, P\_X30170\_at  
 Figure 6840: PRO2569  
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 Figure 6845: PRO23769  
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 Figure 6896A-B: DNA256461, HSAJ6266,  
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 Figure 7036: DNA331114, AF291719, NM\_007182\_at  
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 Figure 7043: PRO83123  
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 Figure 7087: DNA269922, HSISG20GN,  
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 Figure 7138: PRO86782  
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 Figure 7208: PRO49449  
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 Figure 7212: PRO86786  
 Figure 7213: DNA331124, NP\_079430.1, AB018353\_at  
 Figure 7214: PRO86267  
 Figure 7215A-B: DNA330736, AB033044, AB033044\_at  
 Figure 7216A-B: DNA331125, AB037815, AB037815\_at  
 Figure 7217A-B: DNA331898, AF058925, AF058925\_at  
 Figure 7218: PRO86787  
 Figure 7219: DNA331126, AF078867, AF078866\_at  
 Figure 7220: PRO86269  
 Figure 7221: DNA254836, BAA91233.1, AK000529\_at  
 Figure 7222: PRO49931  
 Figure 7223: DNA88277, NP\_006721.1, AK027197\_at  
 Figure 7224: PRO2724  
 Figure 7225: DNA331899, 1399286.1, AW290940\_RC\_at  
 Figure 7226: PRO86788  
 Figure 7227: DNA256872, HSM801990, HSM801990\_at  
 Figure 7228A-B: DNA254192, HUMKIAAK, HUMKIAAK\_at  
 Figure 7229: DNA331900, BIN2, NM\_016293\_at

- Figure 7230: PRO86789  
 Figure 7231A-B: DNA256731, BAA83028.1,  
 AB028999\_at  
 Figure 7232: PRO51665  
 Figure 7233: DNA331901, HSM801036, AB029015\_at  
 Figure 7234A-B: DNA331127, BAA86477.1,  
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 Figure 7235: PRO86270  
 Figure 7236A-B: DNA254672, BAA92652.1,  
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 Figure 7237: PRO49773  
 Figure 7238A-C: DNA331128, NP\_065892.1,  
 AB040884\_at  
 Figure 7239: PRO84841  
 Figure 7240: DNA269976, AAC14260.1,  
 AF039023\_at  
 Figure 7241: PRO58372  
 Figure 7242: DNA331129, HSA227869,  
 HSA227869\_r\_at  
 Figure 7243: DNA256422, HSA227900,  
 HSA227900\_at  
 Figure 7244: DNA331902, BC014522,  
 HSSOM172M\_at  
 Figure 7245: PRO86790  
 Figure 7246: DNA329040, BC001356, HSU72882\_at  
 Figure 7247: PRO84707  
 Figure 7248: DNA331130, AAK50430.1,  
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 Figure 7249: PRO86272  
 Figure 7250A-B: DNA331131, HSA223948,  
 AY013288\_at  
 Figure 7251: DNA326056, NP\_072088.1,  
 AY007810\_at  
 Figure 7252: PRO82491  
 Figure 7253: DNA329041, HSM800399, AF132199\_at  
 Figure 7254: DNA255780, AK022209, AK022209\_at  
 Figure 7255: PRO50835  
 Figure 7256: DNA254922, AK022604, AK022604\_at  
 Figure 7257: PRO50012  
 Figure 7258: DNA330432, FLJ23235, AK026888\_at  
 Figure 7259: PRO85636  
 Figure 7260A-B: DNA256299, AB051489,  
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 Figure 7261: DNA331903, BC015380, HSM801707\_at  
 Figure 7262: DNA255626, HSM802849,  
 HSM802849\_at  
 Figure 7263: PRO50690  
 Figure 7264: DNA331132, NP\_115524.1,  
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 Figure 7265: PRO86273  
 Figure 7266: DNA255964, NP\_079113.1,  
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 Figure 7267: PRO51015  
 Figure 7268: DNA255465, AK024313, AK024313\_at  
 Figure 7269: PRO50532  
 Figure 7270: DNA329597, AK022178, AK022178\_at
- Figure 7271: PRO85129  
 Figure 7272: DNA254228, NP\_079236.1,  
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 Figure 7273: PRO49340  
 Figure 7274: DNA331904, AK023431, AF298880\_at  
 Figure 7275: PRO86791  
 Figure 7276: DNA329078, AF214006, HSM801679\_at  
 Figure 7277: PRO23253  
 Figure 7278: DNA256784, FLJ22104, AK025757\_at  
 Figure 7279: PRO51716  
 Figure 7280: DNA331905, AK001823,  
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 Figure 7281: PRO86792  
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 Figure 7283: PRO84709  
 Figure 7284: DNA331906, HSA227916,  
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 Figure 7285: DNA330023, GADD45A, NM\_001924\_at  
 Figure 7286: PRO85308  
 Figure 7287A-B: DNA272191, RSN, NM\_002956\_at  
 Figure 7288: PRO60456  
 Figure 7289: DNA328418, HUMG0S24A,  
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 Figure 7290: PRO84261  
 Figure 7291: DNA331133, HSU63830, NM\_004180\_at  
 Figure 7292: PRO86274  
 Figure 7293: DNA271310, DUSP8, NM\_004420\_at  
 Figure 7294: PRO59617  
 Figure 7295: DNA331907, AKAP7, NM\_004842\_at  
 Figure 7296: PRO63228  
 Figure 7297: DNA287203, NP\_006182.1,  
 NM\_006191\_at  
 Figure 7298: PRO69487  
 Figure 7299: DNA274783, HSU26424, NM\_006281\_at  
 Figure 7300: PRO62549  
 Figure 7301A-B: DNA255281, NP\_006380.1,  
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 Figure 7302: PRO50357  
 Figure 7303: DNA328712, NP\_006501.1,  
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 Figure 7304: PRO84469  
 Figure 7305: DNA331908, AF161440, NM\_012111\_at  
 Figure 7306: DNA330065, STK18, NM\_014264\_at  
 Figure 7307: PRO85345  
 Figure 7308: DNA152148, DNA152148,  
 HSP1CDC21\_at  
 Figure 7309: PRO10290  
 Figure 7310: DNA329925, HSBP1, NM\_001537\_at  
 Figure 7311: PRO85239  
 Figure 7312: DNA331909, HSCFANT, NM\_002964\_at  
 Figure 7313: PRO86795  
 Figure 7314: DNA329139, NP\_003893.2,  
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 Figure 7315: PRO84774  
 Figure 7316: DNA331910, HSSEC232, NM\_006363\_at

- Figure 7317: PRO86796  
 Figure 7318: DNA329047, BATF, NM\_006399\_at  
 Figure 7319: PRO58425  
 Figure 7320: DNA274167, AF026166, NM\_006431\_at  
 Figure 7321: PRO62097  
 Figure 7322: DNA254572, NP\_006576.1,  
 NM\_006585\_at  
 Figure 7323: PRO49675  
 Figure 7324A-B: DNA331911, AB003334,  
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 Figure 7325: PRO86797  
 Figure 7326: DNA331912, BC009405, NM\_013411\_at  
 Figure 7327: PRO86798  
 Figure 7328: DNA255289, MELK, NM\_014791\_at  
 Figure 7329: PRO50363  
 Figure 7330A-B: DNA331913, BAB21784.1,  
 NM\_015383\_at  
 Figure 7331: PRO86799  
 Figure 7332: DNA329148, LOC51042, NM\_015871\_at  
 Figure 7333: PRO84782  
 Figure 7334: DNA326221, AF125098, NM\_016095\_at  
 Figure 7335: PRO82634  
 Figure 7336: DNA331914, BC009398,  
 HUMP1CDC47\_at  
 Figure 7337: PRO86800  
 Figure 7338A-B: DNA328312, HUMAREB6,  
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 Figure 7339: PRO84180  
 Figure 7340: DNA325941, HSPCA, HSHSP90R\_at  
 Figure 7341: PRO82388  
 Figure 7342: DNA328483, VIT1, NM\_000179\_at  
 Figure 7343: PRO84309  
 Figure 7344: DNA271847, HUMDNAJHOM,  
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 Figure 7345: PRO60127  
 Figure 7346: DNA331915, BC001786, NM\_002014\_at  
 Figure 7347: PRO59262  
 Figure 7348: DNA331916, HUMMIF, NM\_002415\_at  
 Figure 7349: DNA331917, PHF1, NM\_002636\_at  
 Figure 7350: PRO86802  
 Figure 7351: DNA329604, SRP54, NM\_003136\_at  
 Figure 7352: PRO85134  
 Figure 7353A-B: DNA331134, NP\_003381.1,  
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 Figure 7354: PRO86275  
 Figure 7355A-B: DNA290265, ZNF91,  
 NM\_003430\_f\_at  
 Figure 7356: PRO70395  
 Figure 7357A-C: DNA331918, AF009425,  
 NM\_004338\_at  
 Figure 7358: PRO86803  
 Figure 7359: DNA254582, NP\_004626.1,  
 NM\_004635\_at  
 Figure 7360: PRO49685  
 Figure 7361A-B: DNA275334, NP\_112162.1,  
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- Figure 7362: PRO63009  
 Figure 7363: DNA254157, HSU13045, NM\_005254\_at  
 Figure 7364: PRO49271  
 Figure 7365A-B: DNA124122, RBL2, NM\_005611\_at  
 Figure 7366: PRO6323  
 Figure 7367: DNA330776, TOB1, NM\_005749\_at  
 Figure 7368: PRO58014  
 Figure 7369: DNA326980, AF140598, NM\_014248\_at  
 Figure 7370: PRO83289  
 Figure 7371: DNA271608, HUMRSC419,  
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 Figure 7372: PRO59895  
 Figure 7373: DNA272928, HUMORFKG1F,  
 NM\_014764\_at  
 Figure 7374: PRO61012  
 Figure 7375: DNA290235, NP\_057121.1,  
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 Figure 7376: PRO70335  
 Figure 7377: DNA331135, HUMKG1DD,  
 HUMKG1DD\_at  
 Figure 7378A-B: DNA330119, AF226044,  
 HUMKIAAQ\_at  
 Figure 7379: PRO85381  
 Figure 7380: DNA331137, HS24P52,  
 HUMHSP70H\_at  
 Figure 7381: PRO86278  
 Figure 7382A-B: DNA269805, NP\_001263.1,  
 NM\_001272\_at  
 Figure 7383: PRO58209  
 Figure 7384: DNA270689, HSGATA3R,  
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 Figure 7385: PRO59053  
 Figure 7386: DNA331919, HUMCFA, NM\_002965\_at  
 Figure 7387: PRO80648  
 Figure 7388A-B: DNA304800, NP\_004146.1,  
 NM\_004155\_at  
 Figure 7389: PRO69458  
 Figure 7390: DNA273418, AAG01157.1,  
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 Figure 7391: PRO61417  
 Figure 7392: DNA330066, MLLT3, NM\_004529\_at  
 Figure 7393: PRO85346  
 Figure 7394: DNA270733, S46622, NM\_005605\_at  
 Figure 7395: PRO59094  
 Figure 7396: DNA331138, NP\_005997.2,  
 NM\_006006\_at  
 Figure 7397: PRO86279  
 Figure 7398: DNA331139, NP\_006865.1,  
 NM\_006874\_at  
 Figure 7399: PRO81172  
 Figure 7400: DNA331920, AF090950, NM\_015675\_at  
 Figure 7401: PRO84384  
 Figure 7402: DNA329050, MRPS17, NM\_015969\_at  
 Figure 7403: PRO84712  
 Figure 7404A-B: DNA329122, GS3955,  
 NM\_021643\_at

- Figure 7405: PRO84764  
 Figure 7406: DNA331921, 244055.1, AF320911\_at  
 Figure 7407: PRO86804  
 Figure 7408: DNA331922, AK026275, AK026275\_at  
 Figure 7409: PRO86805  
 Figure 7410A-B: DNA254516, AF288399, AF288399\_at  
 Figure 7411: PRO49623  
 Figure 7412: DNA328313, NP\_115579.1, AK025076\_at  
 Figure 7413: PRO84181  
 Figure 7414: DNA327865, NP\_079105.1, AK026315\_at  
 Figure 7415: PRO83806  
 Figure 7416: DNA294813, NP\_444283.1, P\_T67134\_at  
 Figure 7417: PRO70763  
 Figure 7418A-B: DNA254706, AB046851, AB046851\_at  
 Figure 7419: DNA329052, NP\_078801.1, AK026237\_at  
 Figure 7420: PRO84714  
 Figure 7421: DNA256890, BC008988, P\_Z00467\_at  
 Figure 7422: PRO51824  
 Figure 7423: DNA256291, FLJ21032, AK024685\_f\_at  
 Figure 7424: PRO51335  
 Figure 7425: DNA331923, HSUCP2X12, P\_C69111\_at  
 Figure 7426: DNA213665, DNA213665, P\_X30166\_at  
 Figure 7427: PRO35126  
 Figure 7428: DNA331140, 332752.10, AK023798\_at  
 Figure 7429: PRO86280  
 Figure 7430A-B: DNA331141, BAB13420.1, AB046814\_at  
 Figure 7431: PRO86281  
 Figure 7432: DNA331924, BC004932, AK024551\_at  
 Figure 7433: PRO21434  
 Figure 7434A-B: DNA256267, AB046838, AB046838\_at  
 Figure 7435: DNA327954, BAL, P\_D00629\_at  
 Figure 7436: PRO83879  
 Figure 7437: DNA255798, FLJ12377, AK022439\_at  
 Figure 7438: PRO50853  
 Figure 7439: DNA330389, FLJ12888, AK022950\_at  
 Figure 7440: PRO85598  
 Figure 7441: DNA330086, FLJ12973, AK023035\_at  
 Figure 7442: PRO85360  
 Figure 7443: DNA331142, NP\_116325.1, P\_Z98137\_at  
 Figure 7444: PRO51781  
 Figure 7445: DNA329384, BC008502, P\_Z33372\_at  
 Figure 7446: PRO84960  
 Figure 7447A-B: DNA331143, NP\_149075.2, AK022613\_at  
 Figure 7448: PRO86282  
 Figure 7449: DNA331925, 424693.10, AK022231\_at  
 Figure 7450: PRO86806  
 Figure 7451: DNA331144, NP\_078834.1, AK023982\_at  
 Figure 7452: PRO86283  
 Figure 7453A-B: DNA331926, BAB13449.1, AB046843\_at  
 Figure 7454: PRO51258  
 Figure 7455: DNA255197, DNA255197, P\_Z50392\_at  
 Figure 7456: PRO50276  
 Figure 7457: DNA328010, NP\_149016.1, HSM801092\_at  
 Figure 7458: PRO83928  
 Figure 7459: DNA262805, DNA262805, HSM800425\_at  
 Figure 7460: DNA331146, 1400830.1, HUMJNLTRA\_at  
 Figure 7461: PRO86284  
 Figure 7462: DNA328317, cig5, AF026941\_at  
 Figure 7463: PRO69493  
 Figure 7464: DNA331147, NP\_079104.1, AF131768\_at  
 Figure 7465: PRO86285  
 Figure 7466: DNA255770, DNA255770, AK022106\_at  
 Figure 7467A-C: DNA254412, EVIS, AF008915\_at  
 Figure 7468: PRO49522  
 Figure 7469: DNA331148, 978273.10, AK023244\_at  
 Figure 7470: PRO86286  
 Figure 7471: DNA330532, AK026279, AK026279\_at  
 Figure 7472: PRO85719  
 Figure 7473: DNA330388, FLJ23468, AK027121\_at  
 Figure 7474: PRO85597  
 Figure 7475: DNA331927, AK026969, AK026969\_at  
 Figure 7476: PRO86807  
 Figure 7477: DNA330447, FLJ22757, AK026410\_at  
 Figure 7478: PRO85648  
 Figure 7479: DNA324984, FLJ12298, AK022360\_at  
 Figure 7480: PRO81578  
 Figure 7481: DNA331149, 7697327.1, HSM802839\_at  
 Figure 7482: PRO86287  
 Figure 7483A-B: DNA256267, DNA256267, AK023113\_at  
 Figure 7484: PRO51311  
 Figure 7485: DNA331150, BC017725, 1387341.2\_at  
 Figure 7486: PRO86288  
 Figure 7487: DNA257606, DNA257606, 428093.1\_at  
 Figure 7488: DNA258375, AF283301, 413231.5\_at  
 Figure 7489: PRO52516  
 Figure 7490: DNA331928, AK027419, 154551.10\_at  
 Figure 7491: PRO86808  
 Figure 7492: DNA328319, BC019562, 411364.2\_at  
 Figure 7493: DNA290812, DNA290812, 220495.3\_CON\_at  
 Figure 7494: PRO70559  
 Figure 7495: DNA304799, BC022410, 337588.1\_at  
 Figure 7496: PRO52633  
 Figure 7497: DNA257403, DNA257403, 012814.1\_at  
 Figure 7498: DNA304820, NP\_115940.1, 317557.1\_at  
 Figure 7499: PRO47351  
 Figure 7500: DNA331929, BC019246,

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Figure 7501: PRO83338  
Figure 7502: DNA260581, DNA260581, 127987.6.at  
Figure 7503: PRO54507  
Figure 7504: DNA257576, DNA257576, 334945.2.at  
Figure 7505: DNA304819, BC004398, 202113.2.at  
Figure 7506: DNA304794, FBXO30, 222128.1.at  
Figure 7507: PRO71206  
Figure 7508: DNA259323, DNA259323, 022997.1.at  
Figure 7509: PRO53256  
Figure 7510: DNA304796, MED8, 237428.13.at  
Figure 7511: PRO71208  
Figure 7512: DNA259615, DNA259615, 1000203.1.at  
Figure 7513: DNA304805, AK027628, 475113.7.at  
Figure 7514: PRO69531  
Figure 7515: DNA304793, GBP4, 206425.2.at  
Figure 7516: PRO71205  
Figure 7517: DNA331151, 018033.1,  
018033.1.CON\_at  
Figure 7518: PRO86289  
Figure 7519: DNA304068, AK057631, 1091656.1.at  
Figure 7520: PRO71035  
Figure 7521: DNA257714, EPSTI1, 337352.17.at  
Figure 7522: PRO52268  
Figure 7523: DNA304798, NP\_443097.1, 246119.7.at  
Figure 7524: PRO71210  
Figure 7525: DNA258721, DNA258721, 197627.1.at  
Figure 7526A-B: DNA257461, NP\_113607.1,  
086533.1.at  
Figure 7527: PRO52040  
Figure 7528: DNA331152, 1042156.3, 1042156.3.at  
Figure 7529: PRO86290  
Figure 7530: DNA331153, 004052.1, 004052.1.at  
Figure 7531: PRO86291  
Figure 7532: DNA331930, AK054582, 978231.1.at  
Figure 7533: PRO86809  
Figure 7534: DNA259587, DNA259587, 220866.1.at  
Figure 7535: DNA106195, DNA106195, 359193.13.at  
Figure 7536: DNA331154, 212376.1, 212376.1.at  
Figure 7537: PRO86292  
Figure 7538: DNA331155, 112652.1, 112652.1.at  
Figure 7539: PRO86293  
Figure 7540: DNA304806, BC019022, 983343.1.at  
Figure 7541: PRO71215  
Figure 7542: DNA262708, DNA262708,  
118516.1.RC.at  
Figure 7543: DNA259475, DNA259475, 239162.1.at  
Figure 7544: DNA269148, DNA269148, 411192.2.at  
Figure 7545: DNA304817, BC015532, 211436.3.at  
Figure 7546: PRO71224  
Figure 7547: DNA260313, DNA260313, 1098929.1.at  
Figure 7548: PRO54242  
Figure 7549A-B: DNA328325, NP\_061142.1,  
445188.4.at  
Figure 7550: PRO84190  
Figure 7551A-B: DNA304800, SERPINB9,  
354740.1.at  
Figure 7552: PRO69458  
Figure 7553: DNA331156, 118180.1, 118180.1.at  
Figure 7554: PRO86294  
Figure 7555: DNA287659, AK027790, 406833.1.at  
Figure 7556: PRO69903  
Figure 7557: DNA331931, 092555.3, 092555.4.at  
Figure 7558: PRO86810  
Figure 7559: DNA331157, NP\_439896.1, 022541.5.at  
Figure 7560: PRO86295  
Figure 7561: DNA260573, DNA260573, 899597.1.at  
Figure 7562: PRO54499  
Figure 7563: DNA260157, DNA260157, 236833.1.at  
Figure 7564: PRO54086  
Figure 7565: DNA174145, DNA174145, 100083.2.at  
Figure 7566: PRO35770  
Figure 7567: DNA260167, DNA260167, 264556.1.at  
Figure 7568A-B: DNA331932, 239260.1, 239260.1.at  
Figure 7569: PRO86811  
Figure 7570: DNA260031, DNA260031, 161526.1.at  
Figure 7571: DNA258907, DNA258907, 347940.2.at  
Figure 7572: PRO52840  
Figure 7573: DNA257455, DNA257455, 977723.3.at  
Figure 7574: PRO52035  
Figure 7575: DNA304807, BC014978, 005415.2.at  
Figure 7576: PRO71216  
Figure 7577: DNA258864, DNA258864, 331965.1.at  
Figure 7578: DNA304811, 428051.2, 428051.2.at  
Figure 7579: PRO71220  
Figure 7580: DNA257389, FLJ14906, 987098.1.at  
Figure 7581: PRO51974  
Figure 7582: DNA331158, 130352.1, 130352.1.at  
Figure 7583: PRO86296  
Figure 7584: DNA258951, DNA258951, 222361.1.at  
Figure 7585: DNA331159, NP\_077291.1,  
411426.29.at  
Figure 7586: PRO86297  
Figure 7587: DNA257784, DNA257784, 481853.1.at  
Figure 7588: DNA331933, AF272148, 074299.1.at  
Figure 7589: PRO86812

#### BRIEF DESCRIPTION OF THE DRAWINGS

In the list of figures for the present application, specific cDNA sequences which are differentially expressed in differentiated macrophages as compared to normal undifferentiated monocytes are individually identified with a specific alphanumerical designation. These cDNA sequences are differentially expressed in 5 monocytes that are specifically treated as described in Example 1 below. If start and/or stop codons have been identified in a cDNA sequence shown in the attached figures, they are shown in bold and underlined font, and the encoded polypeptide is shown in the next consecutive figure.

The Figures 1-7589 show the nucleic acids of the invention and their encoded PRO polypeptides. Also included, for convenience is a List of Figures, which gives the figure number and the corresponding 10 DNA or PRO number.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

##### I. Definitions

The terms "PRO polypeptide" and "PRO" as used herein and when immediately followed by a numerical designation refer to various polypeptides, wherein the complete designation (i.e., PRO/number) 15 refers to specific polypeptide sequences as described herein. The terms "PRO/number polypeptide" and "PRO/number" wherein the term "number" is provided as an actual numerical designation as used herein encompass native sequence polypeptides and polypeptide variants (which are further defined herein). The PRO polypeptides described herein may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods. The term "PRO 20 polypeptide" refers to each individual PRO/number polypeptide disclosed herein. All disclosures in this specification which refer to the "PRO polypeptide" refer to each of the polypeptides individually as well as jointly. For example, descriptions of the preparation of, purification of, derivation of, formation of antibodies to or against, administration of, compositions containing, treatment of a disease with, etc., pertain to each polypeptide of the invention individually. The term "PRO polypeptide" also includes variants of the 25 PRO/number polypeptides disclosed herein.

A "native sequence PRO polypeptide" comprises a polypeptide having the same amino acid sequence as the corresponding PRO polypeptide derived from nature. Such native sequence PRO polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence PRO polypeptide" specifically encompasses naturally-occurring truncated or secreted 30 forms of the specific PRO polypeptide (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In various embodiments of the invention, the native sequence PRO polypeptides disclosed herein are mature or full-length native sequence polypeptides comprising the full-length amino acids sequences shown in the accompanying figures. Start and stop codons are shown in bold font and underlined in the figures. 35 However, while the PRO polypeptide disclosed in the accompanying figures are shown to begin with methionine residues designated herein as amino acid position 1 in the figures, it is conceivable and possible that other methionine residues located either upstream or downstream from the amino acid position 1 in the figures may be employed as the starting amino acid residue for the PRO polypeptides.

The PRO polypeptide "extracellular domain" or "ECD" refers to a form of the PRO polypeptide 40 which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a PRO polypeptide

ECD will have less than 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than 0.5% of such domains. It will be understood that any transmembrane domains identified for the PRO polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary  
5 but most likely by no more than about 5 amino acids at either end of the domain as initially identified herein. Optionally, therefore, an extracellular domain of a PRO polypeptide may contain from about 5 or fewer amino acids on either side of the transmembrane domain/extracellular domain boundary as identified in the Examples or specification and such polypeptides, with or without the associated signal peptide, and nucleic acid encoding them, are contemplated by the present invention.

10 The approximate location of the "signal peptides" of the various PRO polypeptides disclosed herein are shown in the present specification and/or the accompanying figures. It is noted, however, that the C-terminal boundary of a signal peptide may vary, but most likely by no more than about 5 amino acids on either side of the signal peptide C-terminal boundary as initially identified herein, wherein the C-terminal boundary of the signal peptide may be identified pursuant to criteria routinely employed in the art for  
15 identifying that type of amino acid sequence element (e.g., Nielsen et al., Prot. Eng. 10:1-6 (1997) and von Heinje et al., Nucl. Acids. Res. 14:4683-4690 (1986)). Moreover, it is also recognized that, in some cases, cleavage of a signal sequence from a secreted polypeptide is not entirely uniform, resulting in more than one secreted species. These mature polypeptides, where the signal peptide is cleaved within no more than about 5 amino acids on either side of the C-terminal boundary of the signal peptide as identified herein, and the  
20 polynucleotides encoding them, are contemplated by the present invention.

"PRO polypeptide variant" means an active PRO polypeptide as defined above or below having at least about 80% amino acid sequence identity with a full-length native sequence PRO polypeptide sequence as disclosed herein, a PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any  
25 other fragment of a full-length PRO polypeptide sequence as disclosed herein. Such PRO polypeptide variants include, for instance, PRO polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. Ordinarily, a PRO polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81% amino acid sequence identity, alternatively at least about 82% amino acid sequence identity,  
30 alternatively at least about 83% amino acid sequence identity, alternatively at least about 84% amino acid sequence identity, alternatively at least about 85% amino acid sequence identity, alternatively at least about 86% amino acid sequence identity, alternatively at least about 87% amino acid sequence identity, alternatively at least about 88% amino acid sequence identity, alternatively at least about 89% amino acid sequence identity, alternatively at least about 90% amino acid sequence identity, alternatively at least about  
35 91% amino acid sequence identity, alternatively at least about 92% amino acid sequence identity, alternatively at least about 93% amino acid sequence identity, alternatively at least about 94% amino acid sequence identity, alternatively at least about 95% amino acid sequence identity, alternatively at least about 96% amino acid sequence identity, alternatively at least about 97% amino acid sequence identity, alternatively at least about 98% amino acid sequence identity and alternatively at least about 99% amino  
40 acid sequence identity to a full-length native sequence PRO polypeptide sequence as disclosed herein, a

PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other specifically defined fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, PRO variant polypeptides are at least about 10 amino acids in length, alternatively at least about 20 amino acids in length,  
5 alternatively at least about 30 amino acids in length, alternatively at least about 40 amino acids in length, alternatively at least about 50 amino acids in length, alternatively at least about 60 amino acids in length, alternatively at least about 70 amino acids in length, alternatively at least about 80 amino acids in length, alternatively at least about 90 amino acids in length, alternatively at least about 100 amino acids in length, alternatively at least about 150 amino acids in length, alternatively at least about 200 amino acids in length,  
10 alternatively at least about 300 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the PRO polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific PRO polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not  
15 considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of  
20 the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S.  
25 Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:  
30

$$100 \text{ times the fraction } X/Y$$

35 where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations using this  
40

method, Tables 2 and 3 demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO", wherein "PRO" represents the amino acid sequence of a hypothetical PRO polypeptide of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, and "X", "Y" and "Z" each represent different hypothetical amino acid residues.

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % amino acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % amino acid sequence identity value is determined by dividing (a) the number of matching identical amino acid residues between the amino acid sequence of the PRO polypeptide of interest having a sequence derived from the native PRO polypeptide and the comparison amino acid sequence of interest (i.e., the sequence against which the PRO polypeptide of interest is being compared which may be a PRO variant polypeptide) as determined by WU-BLAST-2 by (b) the total number of amino acid residues of the PRO polypeptide of interest. For example, in the statement "a polypeptide comprising an the amino acid sequence A which has or having at least 80% amino acid sequence identity to the amino acid sequence B", the amino acid sequence A is the comparison amino acid sequence of interest and the amino acid sequence B is the amino acid sequence of the PRO polypeptide of interest.

Percent amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the

length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

"PRO variant polynucleotide" or "PRO variant nucleic acid sequence" means a nucleic acid molecule which encodes an active PRO polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal peptide, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Ordinarily, a PRO variant polynucleotide will have at least about 80% nucleic acid sequence identity, alternatively at least about 81% nucleic acid sequence identity, alternatively at least about 82% nucleic acid sequence identity, alternatively at least about 83% nucleic acid sequence identity, alternatively at least about 84% nucleic acid sequence identity, alternatively at least about 85% nucleic acid sequence identity, alternatively at least about 86% nucleic acid sequence identity, alternatively at least about 87% nucleic acid sequence identity, alternatively at least about 88% nucleic acid sequence identity, alternatively at least about 89% nucleic acid sequence identity, alternatively at least about 90% nucleic acid sequence identity, alternatively at least about 91% nucleic acid sequence identity, alternatively at least about 92% nucleic acid sequence identity, alternatively at least about 93% nucleic acid sequence identity, alternatively at least about 94% nucleic acid sequence identity, alternatively at least about 95% nucleic acid sequence identity, alternatively at least about 96% nucleic acid sequence identity, alternatively at least about 97% nucleic acid sequence identity, alternatively at least about 98% nucleic acid sequence identity and alternatively at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding a full-length native sequence PRO polypeptide sequence as disclosed herein, a full-length native sequence PRO polypeptide sequence lacking the signal peptide as disclosed herein, an extracellular domain of a PRO polypeptide, with or without the signal sequence, as disclosed herein or any other fragment of a full-length PRO polypeptide sequence as disclosed herein. Variants do not encompass the native nucleotide sequence.

Ordinarily, PRO variant polynucleotides are at least about 30 nucleotides in length, alternatively at least about 60 nucleotides in length, alternatively at least about 90 nucleotides in length, alternatively at least about 120 nucleotides in length, alternatively at least about 150 nucleotides in length, alternatively at least about 180 nucleotides in length, alternatively at least about 210 nucleotides in length, alternatively at least about 240 nucleotides in length, alternatively at least about 270 nucleotides in length, alternatively at least about 300 nucleotides in length, alternatively at least about 450 nucleotides in length, alternatively at least about 600 nucleotides in length, alternatively at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to PRO-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the PRO nucleic acid sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code

for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech,  
5 Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence  
10 D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

$$100 \text{ times the fraction } W/Z$$

15 where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5,  
20 demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA", wherein "PRO-DNA" represents a hypothetical PRO-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides.

25 Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program. However, % nucleic acid sequence identity values may also be obtained as described below by using the WU-BLAST-2 computer program (Altschul et al., *Methods in Enzymology* 266:460-480 (1996)). Most of the WU-BLAST-2 search parameters are set to the default values. Those not set to default values, i.e., the  
30 adjustable parameters, are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11, and scoring matrix = BLOSUM62. When WU-BLAST-2 is employed, a % nucleic acid sequence identity value is determined by dividing (a) the number of matching identical nucleotides between the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest having a sequence derived from the native sequence PRO polypeptide-encoding nucleic acid and the comparison  
35 nucleic acid molecule of interest (i.e., the sequence against which the PRO polypeptide-encoding nucleic acid molecule of interest is being compared which may be a variant PRO polynucleotide) as determined by WU-BLAST-2 by (b) the total number of nucleotides of the PRO polypeptide-encoding nucleic acid molecule of interest. For example, in the statement "an isolated nucleic acid molecule comprising a nucleic acid sequence A which has or having at least 80% nucleic acid sequence identity to the nucleic acid  
40 sequence B", the nucleic acid sequence A is the comparison nucleic acid molecule of interest and the nucleic

acid sequence B is the nucleic acid sequence of the PRO polypeptide-encoding nucleic acid molecule of interest.

Percent nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institute of Health, Bethesda, MD. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

10 In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

where W is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

In other embodiments, PRO variant polynucleotides are nucleic acid molecules that encode an active PRO polypeptide and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length PRO polypeptide as disclosed herein. PRO variant polypeptides may be those that are encoded by a PRO variant polynucleotide.

"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the PRO polypeptide natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

An "isolated" PRO polypeptide-encoding nucleic acid or other polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. An isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from the

specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

5       The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

10      Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, 15     in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

20      The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polyepitopic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

25      "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The 30     higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

35      "Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl,

0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

5 "Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above. An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

10

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a PRO 15 polypeptide fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody 20 does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin 25 constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

30 "Active" or "activity" for the purposes herein refers to form(s) of a PRO polypeptide which retain a biological and/or an immunological activity of native or naturally-occurring PRO, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring PRO other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO and an "immunological" activity refers to the ability to 35 induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring PRO.

40 The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native PRO polypeptide disclosed herein. In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics a biological activity of a native PRO polypeptide disclosed herein. Suitable agonist or antagonist molecules

specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, antisense oligonucleotides, small organic molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

5 "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

10 "Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

15 "Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

20 "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as 25 polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

30 "Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

35 Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')<sub>2</sub> fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

40 "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V<sub>H</sub>-V<sub>L</sub> dimer. Collectively, the six CDRs confer antigen-

binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

10 The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: 15 IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the V<sub>H</sub> and V<sub>L</sub> domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired 20 structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) in the same polypeptide chain (V<sub>H</sub>-V<sub>L</sub>). By using a linker that is too short to allow pairing between the two 25 domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials 30 which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE 35 under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

The word "label" when used herein refers to a detectable compound or composition which is 5 conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can 10 adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

15 A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

20 The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to a morbidity in the mammal. Also included are diseases in which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, etc.

25 The term "T cell mediated disease" means a disease in which T cells directly or indirectly mediate or otherwise contribute to a morbidity in a mammal. The T cell mediated disease may be associated with cell mediated effects, lymphokine mediated effects, etc., and even effects associated with B cells if the B cells are stimulated, for example, by the lymphokines secreted by T cells.

30 Examples of immune-related and inflammatory diseases, some of which are immune or T cell mediated, which can be treated according to the invention include systemic lupus erythematosis, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), 35 autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E 40 and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis,

granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic 5 diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease. Infectious diseases including viral diseases such as AIDS (HIV infection), hepatitis A, B, C, D, and E, herpes, etc., bacterial infections, fungal infections, protozoal infections and parasitic infections.

The term "effective amount" is a concentration or amount of a PRO polypeptide and/or 10 agonist/antagonist which results in achieving a particular stated purpose. An "effective amount" of a PRO polypeptide or agonist or antagonist thereof may be determined empirically. Furthermore, a "therapeutically effective amount" is a concentration or amount of a PRO polypeptide and/or agonist/antagonist which is effective for achieving a stated therapeutic effect. This amount may also be determined empirically.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function 15 of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup> and Re<sup>186</sup>), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples 20 of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, e.g., paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, France), toxotere, methotrexate, cisplatin, melphalan, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), melphalan and other related nitrogen 25 mustards. Also included in this definition are hormonal agents that act to regulate or inhibit hormone action on tumors such as tamoxifen and onapristone.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits 30 growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, 35 mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

The term "cytokine" is a generic term for proteins released by one cell population which act on 40 another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and

traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- $\alpha$  and - $\beta$ ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- $\beta$ ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- $\alpha$  and TGF- $\beta$ ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- $\alpha$ , - $\beta$ , and - $\gamma$ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 $\alpha$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF- $\alpha$  or TNF- $\beta$ ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

As used herein, the term "inflammatory cells" designates cells that enhance the inflammatory response such as mononuclear cells, eosinophils, macrophages, and polymorphonuclear neutrophils (PMN).

**Table 1**

45

50

55

Table 1 (cont')

```

/*
 */
#include <stdio.h>
5 #include <ctype.h>

#define MAXJMP 16 /* max jumps in a diag */
#define MAXGAP 24 /* don't continue to penalize gaps larger than this */
#define J MPS 1024 /* max jmps in a path */
10 #define MX 4 /* save if there's at least MX-1 bases since last jmp */

#define DMAT 3 /* value of matching bases */
#define DMIS 0 /* penalty for mismatched bases */
#define DINS0 8 /* penalty for a gap */
15 #define DINS1 1 /* penalty per base */
#define PINS0 8 /* penalty for a gap */
#define PINS1 4 /* penalty per residue */

20 struct jmp {
    short n[MAXJMP]; /* size of jmp (neg for delay) */
    unsigned short x[MAXJMP]; /* base no. of jmp in seq x */
}; /* limits seq to 2^16 -1 */

25 struct diag {
    int score; /* score at last jmp */
    long offset; /* offset of prev block */
    short ijmp; /* current jmp index */
    struct jmp jp; /* list of jmps */
};

30 struct path {
    int spc; /* number of leading spaces */
    short n[J MPS]; /* size of jmp (gap) */
    int x[J MPS]; /* loc of jmp (last elem before gap) */
};

35 char *ofile; /* output file name */
char *namex[2]; /* seq names: getseqs() */
char *prog; /* prog name for err msgs */
40 char *seqx[2]; /* seqs: getseqs() */
int dmax; /* best diag: nw0 */
int dmax0; /* final diag */
int dna; /* set if dna: main0 */
int endgaps; /* set if penalizing end gaps */
45 int gapx, gapy; /* total gaps in seqs */
int len0, len1; /* seq lens */
int ngapx, ngapy; /* total size of gaps */
int smax; /* max score: nw0 */
int *xbm; /* bitmap for matching */
50 long offset; /* current offset in jmp file */
struct diag *dx; /* holds diagonals */
struct path pp[2]; /* holds path for seqs */

55 char *calloc0, *malloc0, *index0, *strcpy0;
char *getseq0, *g_malloc0;

```

**Table 1 (cont')**

```

/* Needleman-Wunsch alignment program
 *
 * usage: progs file1 file2
5   * where file1 and file2 are two dna or two protein sequences.
 * The sequences can be in upper- or lower-case and may contain ambiguity
 * Any lines beginning with ';' or '<' are ignored
 * Max file length is 65535 (limited by unsigned short x in the jmp struct)
 * A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
10  * Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
15  #include "nw.h"
  #include "day.h"

  static _dbval[26] = {
20    1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

  static _pbval[26] = {
25    1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
      128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
      1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
      1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)
30  main
    int     ac;
    char   *av[ ];
{
    prog = av[0];
    if (ac != 3) {
        fprintf(stderr, "usage: %s file1 file2\n", prog);
        fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
        fprintf(stderr, "The sequences can be in upper- or lower-case\n");
        fprintf(stderr, "Any lines beginning with ';' or '<' are ignored\n");
        fprintf(stderr, "Output is in the file 'align.out'\n");
        exit(1);
    }
    namex[0] = av[1];
    namex[1] = av[2];
45    seqx[0] = getseq(namex[0], &len0);
    seqx[1] = getseq(namex[1], &len1);
    xbm = (dna)?_dbval :_pbval;

    endgaps = 0;           /* 1 to penalize endgaps */
50    ofile = "align.out"; /* output file */

    nw();                 /* fill in the matrix, get the possible jmps */
    readjmps();           /* get the actual jmps */
    print();               /* print stats, alignment */
55    cleanup();           /* unlink any tmp files */
}

```

**Table 1 (cont')**

```

/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
5   * When scores are equal, we prefer mismatches to any gap, prefer
   * a new gap to extending an ongoing gap, and prefer a gap in seqx
   * to a gap in seq y.
   */
nw0

10  nw
{
    char      *px, *py;      /* seqs and ptrs */
    int       *ndely, *dely;  /* keep track of dely */
    int       ndelx, delx;   /* keep track of delx */
    int       *tmp;
    int       mis;           /* for swapping row0, row1 */
    int       ins0, ins1;    /* score for each type */
    register  id;           /* insertion penalties */
    register  ij;           /* diagonal index */
    register  *col0, *col1;  /* jmp index */
    register  xx, yy;       /* score for curr, last row */
                           /* index into seqs */

    dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

25  ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
    dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
    col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
    col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
    ins0 = (dna)? DINS0 : PINS0;
30  ins1 = (dna)? DINS1 : PINS1;

    smax = -10000;
    if (endgaps) {
        for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
            col0[yy] = dely[yy] = col0[yy-1] - ins1;
            ndely[yy] = yy;
        }
        col0[0] = 0;          /* Waterman Bull Math Biol 84 */
    }
40  else
        for (yy = 1; yy <= len1; yy++)
            dely[yy] = -ins0;

    /* fill in match matrix
     */
45  for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
        /* initialize first entry in col
         */
        if (endgaps) {
            if (xx == 1)
                col1[0] = delx = -(ins0+ins1);
            else
                col1[0] = delx = col0[0] - ins1;
                ndelx = xx;
55        }
        else {
            col1[0] = 0;
            delx = -ins0;
            ndelx = 0;
60        }
    }
}

```

**Table 1 (cont')**

...nw

```

for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
    mis = col0[yy-1];
    if (dna)
        mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
    else
        mis += _day[*px-'A'][*py-'A'];

    /* update penalty for del in x seq;
     * favor new del over ongoing del
     * ignore MAXGAP if weighting endgaps
     */
    if (endgaps || ndely[yy] < MAXGAP) {
        if (col0[yy] - ins0 >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else {
            dely[yy] -= ins1;
            ndely[yy]++;
        }
    } else {
        if (col0[yy] - (ins0+ins1) >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else
            ndely[yy]++;
    }

    /* update penalty for del in y seq;
     * favor new del over ongoing del
     */
    if (endgaps || ndelx < MAXGAP) {
        if (col1[yy-1] - ins0 >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else {
            delx -= ins1;
            ndelx++;
        }
    } else {
        if (col1[yy-1] - (ins0+ins1) >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else
            ndelx++;
    }

    /* pick the maximum score; we're favoring
     * mis over any del and delx over dely
     */
}

```

55

60

Table 1 (cont')

...nw

```

5      id = xx - yy + len1 - 1;
       if (mis >= delx && mis >= dely[yy])
          col1[yy] = mis;
       else if (delx >= dely[yy]) {
          col1[yy] = delx;
          ij = dx[id].ijmp;
          if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
10         && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
             dx[id].jmp++; 
             if (++ij >= MAXJMP) {
                writejmps(id);
                ij = dx[id].ijmp = 0;
                dx[id].offset = offset;
                offset += sizeof(struct jmp) + sizeof(offset);
             }
          }
          dx[id].jp.n[ij] = nodelx;
          dx[id].jp.x[ij] = xx;
          dx[id].score = delx;
       }
20     else {
          col1[yy] = dely[yy];
          ij = dx[id].ijmp;
          if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
25         && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
             dx[id].jmp++;
             if (++ij >= MAXJMP) {
                writejmps(id);
                ij = dx[id].ijmp = 0;
                dx[id].offset = offset;
                offset += sizeof(struct jmp) + sizeof(offset);
             }
          }
          dx[id].jp.n[ij] = -ndely[yy];
          dx[id].jp.x[ij] = xx;
          dx[id].score = dely[yy];
       }
30     }
35     if (xx == len0 && yy < len1) {
          /* last col
          */
          if (endgaps)
             col1[yy] -= ins0+ins1*(len1-yy);
40     if (col1[yy] > smax) {
             smax = col1[yy];
             dmax = id;
          }
45     }
50     if (endgaps && xx < len0)
             col1[yy-1] -= ins0+ins1*(len0-xx);
      if (col1[yy-1] > smax) {
             smax = col1[yy-1];
             dmax = id;
55     }
      tmp = col0; col0 = col1; col1 = tmp;
   }
60   (void) free((char *)ndely);
   (void) free((char *)dely);
   (void) free((char *)col0);
   (void) free((char *)col1);
}

```

**Table 1 (cont')**

```

/*
 *
 * print() -- only routine visible outside this module
5   *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[ ]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
10  *
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() - put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */
15
#include "nw.h"

#define SPC      3
#define P_LINE   256     /* maximum output line */
20 #define P_SPC   3     /* space between name or num and seq */

extern _day[26][26];
int    olen;           /* set output line length */
FILE   *fx;            /* output file */

25
print()
{
    int     lx, ly, firstgap, lastgap;      /* overlap */
30
    if ((fx = fopen(ofile, "w")) == 0) {
        fprintf(stderr, "%s: can't write %s\n", prog, ofile);
        cleanup(1);
    }
35
    fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
    fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
    olen = 60;
    lx = len0;
    ly = len1;
40
    firstgap = lastgap = 0;
    if (dmax < len1 - 1) {          /* leading gap in x */
        pp[0].spc = firstgap = len1 - dmax - 1;
        ly -= pp[0].spc;
    }
45
    else if (dmax > len1 - 1) {    /* leading gap in y */
        pp[1].spc = firstgap = dmax - (len1 - 1);
        lx -= pp[1].spc;
    }
50
    if (dmax0 < len0 - 1) {        /* trailing gap in x */
        lastgap = len0 - dmax0 - 1;
        lx -= lastgap;
    }
    else if (dmax0 > len0 - 1) {  /* trailing gap in y */
        lastgap = dmax0 - (len0 - 1);
        ly -= lastgap;
    }
55
    getmat(lx, ly, firstgap, lastgap);
    pr_align();
}
60
}

```

Table 1 (cont')

```

/*
 * trace back the best path, count matches
 */
5 static
getmat(lx, ly, firstgap, lastgap) getmat
    int      lx, ly;          /* "core" (minus endgaps) */
    int      firstgap, lastgap; /* leading/trailing overlap */
{
10   int      nm, i0, i1, siz0, siz1;
    char     outx[32];
    double   pct;
    register int n0, n1;
    register char *p0, *p1;
15   /* get total matches, score
 */
    i0 = i1 = siz0 = siz1 = 0;
    p0 = seqx[0] + pp[1].spc;
20   p1 = seqx[1] + pp[0].spc;
    n0 = pp[1].spc + 1;
    n1 = pp[0].spc + 1;

25   nm = 0;
    while (*p0 && *p1) {
        if (siz0) {
            p1++;
            n1++;
            siz0--;
40        }
        else if (siz1) {
            p0++;
            n0++;
            siz1--;
50        }
        else {
            if (xbm[*p0-'A']&xbm[*p1-'A'])
                nm++;
            if (n0++ == pp[0].x[i0])
                siz0 = pp[0].n[i0++];
            if (n1++ == pp[1].x[i1])
                siz1 = pp[1].n[i1++];
            p0++;
            p1++;
45        }
    }

    /* pct homology:
     * if penalizing endgaps, base is the shorter seq
     * else, knock off overhangs and take shorter core
     */
55   if (endgaps)
        lx = (len0 < len1)? len0 : len1;
    else
        lx = (lx < ly)? lx : ly;
    pct = 100.*(double)nm/(double)lx;
    fprintf(fx, "%u";
    fprintf(fx, "<%d match%ls in an overlap of %d: %.2f percent similarity\n",
    nm, (nm == 1)? "" : "es", lx, pct);
60

```

**Table 1 (cont')**

```

5      sprintf(fx, "<gaps in first sequence: %d", gapx);           ...getmat
if (gapx) {
    (void) sprintf(outx, " (%d %s%$)", ngapx, (dna)? "base": "residue", (ngapx == 1)? ":" : "s");
    fprintf(fx, "%s", outx);

10     sprintf(fx, ", gaps in second sequence: %d", gapy);
if (gapy) {
    (void) sprintf(outx, " (%d %s%$)", ngapy, (dna)? "base": "residue", (ngapy == 1)? ":" : "s");
    fprintf(fx, "%s", outx);
}
15     if (dna)
        fprintf(fx,
            "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
            smax, DMAT, DMIS, DINS0, DINS1);
    else
        fprintf(fx,
            "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
            smax, PINS0, PINS1);
if (endgaps)
    fprintf(fx,
25        "<endgaps penalized. left endgap: %d %s%$, right endgap: %d %s%$",
        firstgap, (dna)? "base" : "residue", (firstgap == 1)? ":" : "s",
        lastgap, (dna)? "base" : "residue", (lastgap == 1)? ":" : "s");
else
    fprintf(fx, "<endgaps not penalized\n");
30 }
static nm;          /* matches in core -- for checking */
static lmax;        /* lengths of stripped file names */
static ij[2];        /* jmp index for a path */
static nc[2];        /* number at start of current line */
35 static ni[2];        /* current elem number -- for gapping */
static siz[2];
static char *ps[2];    /* ptr to current element */
static char *po[2];    /* ptr to next output char slot */
static char out[2][P_LINE]; /* output line */
40 static char star[P_LINE]; /* set by stars() */

/*
 * print alignment of described in struct path pp[ ]
 */
45 static
pr_align()
{
    int nn;          /* char count */
    int more;
50    register i;

    for (i = 0, lmax = 0; i < 2; i++) {
        nn = stripname(name[i]);
        if (nn > lmax)
            lmax = nn;

        nc[i] = 1;
        ni[i] = 1;
        siz[i] = ij[i] = 0;
        ps[i] = seqx[i];
        po[i] = out[i];
55    }
}
60

```

**pr\_align**

Table 1 (cont')

```

for (nn = nm = 0, more = 1; more; ) {
    for (i = more = 0; i < 2; i++) {
        /*
         * do we have more of this sequence?
         */
        if (!*ps[i])
            continue;
        more++;
        if (pp[i].spc) { /* leading space */
            *po[i]++ = ' ';
            pp[i].spc--;
        }
        else if (siz[i]) { /* in a gap */
            *po[i]++ = '-';
            siz[i]--;
        }
        else { /* we're putting a seq element */
            *po[i] = *ps[i];
            if (islower(*ps[i]))
                *ps[i] = toupper(*ps[i]);
            po[i]++;
            ps[i]++;
            /*
             * are we at next gap for this seq?
             */
            if (ni[i] == pp[i].x[ij[i]]) {
                /*
                 * we need to merge all gaps
                 * at this location
                 */
                siz[i] = pp[i].n[ij[i]++];
                while (ni[i] == pp[i].x[ij[i]])
                    siz[i] += pp[i].n[ij[i]++];
            }
            ni[i]++;
        }
    }
    if (++nn == olen || !more && nn) {
        dumpblock();
        for (i = 0; i < 2; i++)
            po[i] = out[i];
        nn = 0;
    }
}
/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()
{
    register i;
    for (i = 0; i < 2; i++)
        *po[i] = '\0';
}

```

**Table 1 (cont')****...dumpblock**

```

5   (void) putc('\n', fx);
for (i = 0; i < 2; i++) {
    if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
        if (i == 0)
            nums(i);
        if (i == 0 && *out[1])
            stars0;
        putline(ix);
        if (i == 0 && *out[1])
            fprintf(fx, star);
        if (i == 1)
            nums(i);
    }
}
10
15
20 /* * put out a number line: dumpblock()
 */
static
25     nums(ix)
{
    int      ix;      /* index in out[ ] holding seq line */
    char      nline[P_LINE];
    register  i, j;
    register char  *pn, *px, *py;
30
35     for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
        *pn = ' ';
     for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
        if (*py == ' ' || *py == '-')
            *pn = ' ';
        else {
            if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                j = (i < 0)? -i : i;
                for (px = pn; j; j /= 10, px--)
                    *px = j%10 + '0';
                if (i < 0)
                    *px = '-';
            }
            else
                *pn = ' ';
            i++;
        }
    }
50
55     *pn = '\0';
     nc[ix] = i;
     for (pn = nline; *pn; pn++)
        (void) putc(*pn, fx);
        (void) putc('\n', fx);
}
60
65 /* * put out a line (name, [num], seq, [num]): dumpblock()
 */
static
putline(ix)
int      ix;
{

```

**nums**

**putline**

**Table 1 (cont')****...putline**

```

5      int          i;
register char    *px;

for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
    (void) putc(*px, fx);
for (; i < lmax+P_SPC; i++)
    (void) putc(' ', fx);

10     /* these count from 1:
        * ni[ ] is current element (from 1)
        * nc[ ] is number at start of current line
        */
15     for (px = out[ix]; *px; px++)
        (void) putc(*px&0x7F, fx);
    (void) putc('\n', fx);
}

20     /*
        * put a line of stars (seqs always in out[0], out[1]): dumpblock()
        */
25     static
stars() {
    stars
    {
30         int          i;
register char    *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ') ||
           !*out[1] || (*out[1] == ' ' && *(po[1]) == ') )
            return;
35         px = star;
        for (i = lmax+P_SPC; i; i--)
            *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
40             if (isalpha(*p0) && isalpha(*p1)) {

                if (xbm[*p0-'A']&xbm[*p1-'A']) {
                    cx = '*';
                    nm++;
45                }
                else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                    cx = ':';
                else
                    cx = ' ';
50            }
            else
                cx = ' ';
            *px++ = cx;
        }
55         *px++ = '\n';
        *px = '\0';
    }
}

```

60

**Table 1 (cont')**

```
/*
 * strip path or prefix from pn, return len: pr_align()
 */
5 static
stripname(pn)
    char    *pn;      /* file name (may be path) */
{
10    register char   *px, *py;

    py = 0;
    for (px = pn; *px; px++)
        if (*px == '/')
15        py = px + 1;
    if (py)
        (void) strcpy(pn, py);
    return(strlen(pn));
20 }
```

25

30

35

40

45

50

55

60

**Table 1 (cont')**

```

/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_calloc() -- calloc() with error checkin
 * readjmps() -- get the good jmps, from tmp file if necessary
 * writejmps() -- write a filled array of jmps to a tmp file: nw()
 */
5   #include "nw.h"
10  #include <sys/file.h>

    char *jname = "/tmp/homgXXXXXX";           /* tmp file for jmps */
    FILE *fj;

15  int long cleanup();                      /* cleanup tmp file */

20  /*
21   * remove any tmp file if we blow
22   */
23  cleanup(i)
24  {
25      int i;
26
27      if (fj)
28          (void) unlink(jname);
29      exit(i);
30  }

31  /*
32   * read, return ptr to seq, set dna, len, maxlen
33   * skip lines starting with ';', '<', or '>'
34   * seq in upper or lower case
35   */
36  char *
37  getseq(file, len)
38
39      char *file;    /* file name */
40      int *len;     /* seq len */
41
42      char line[1024], *pseq;
43      register char *px, *py;
44      int natgc, tlen;
45      FILE *fp;
46
47      if ((fp = fopen(file, "r")) == 0) {
48          fprintf(stderr, "%s: can't read %s\n", prog, file);
49          exit(1);
50      }
51      tlen = natgc = 0;
52      while (fgets(line, 1024, fp)) {
53          if (*line == ';' || *line == '<' || *line == '>')
54              continue;
55          for (px = line; *px != '\n'; px++)
56              if (isupper(*px) || islower(*px))
57                  tlen++;
58      }
59      if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
60          fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
61          exit(1);
62      }
63      pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';

```

cleanup  
getseq

**Table 1 (cont')**

```

...getseq
5      py = pseq + 4;
      *len = tlen;
      rewind(fp);

10     while (fgets(line, 1024, fp)) {
          if (*line == ';' || *line == '<' || *line == '>')
              continue;
          for (px = line; *px != '\n'; px++) {
              if (isupper(*px))
                  *py++ = *px;
              else if (islower(*px))
                  *py++ = toupper(*px);
15          if (index("ATGCU", *(py-1)))
              natgc++;
          }
          *py++ = '\0';
20          *py = '\0';
          (void) fclose(fp);
          dna = natgc > (tlen/3);
          return(pseq+4);
      }

25      char *
      g_calloc(msg, nx, sz)
          char    *msg;           /* program, calling routine */
          int     nx, sz;         /* number and size of elements */
30      {
          char    *px, *calloc();
          if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
              if (*msg) {
                  fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n",
35                  prog, msg, nx, sz);
                  exit(1);
              }
          }
          return(px);
40      }

        /*
         * get final jmps from dx[ ] or tmp file, set pp[ ], reset dmax: main()
         */
45      readjmps()
          readjmps
{
        int             fd = -1;
        int             siz, i0, i1;
50      register i, j, xx;

        if (fj) {
            (void) fclose(fj);
            if ((fd = open(jname, O_RDONLY, 0)) < 0) {
                fprintf(stderr, "%s: can't open() %s\n", prog, jname);
                cleanup(1);
            }
        }
        for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
            while (1) {
                for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                    ;
            }
        }
    }

```

**Table 1 (cont')****...readjmps**

```

5           if (j < 0 && dx[dmax].offset && fj) {
6               (void) lseek(fd, dx[dmax].offset, 0);
7               (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
8               (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
9               dx[dmax].ijmp = MAXJMP-1;
10          }
11      else
12          break;
13  }
14  if (i >= JMPS) {
15      fprintf(stderr, "%s: too many gaps in alignment\n", prog);
16      cleanup(1);
17  }
18  if (j >= 0) {
19      siz = dx[dmax].jp.n[j];
20      xx = dx[dmax].jp.x[j];
21      dmax += siz;
22      if (siz < 0) { /* gap in second seq */
23          pp[1].n[i1] = -siz;
24          xx += siz;
25          /* id = xx - yy + len1 - 1
26          */
27          pp[1].x[i1] = xx - dmax + len1 - 1;
28          gapy++;
29          ngapy -= siz;
30      /* ignore MAXGAP when doing endgaps */
31      siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
32      i1++;
33      }
34      else if (siz > 0) { /* gap in first seq */
35          pp[0].n[i0] = siz;
36          pp[0].x[i0] = xx;
37          gapx++;
38          ngapx += siz;
39      /* ignore MAXGAP when doing endgaps */
40      siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
41      i0++;
42      }
43      }
44      else
45          break;
46  }
47  /* reverse the order of jmps
48  */
49  for (j = 0, i0--; j < i0; j++, i0--) {
50      i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
51      i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
52  }
53  for (j = 0, i1--; j < i1; j++, i1--) {
54      i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
55      i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
56  }
57  if (fd >= 0)
58      (void) close(fd);
59  if (fj) {
60      (void) unlink(jname);
61      fj = 0;
62      offset = 0;
63  }

```

**Table 1 (cont?)**

```

/*
 * write a filled jmp struct offset of the prev one (if any): nw0
 */
5   writejmps(ix)
    writejmps
    int      ix;
    {
10   char     *mktemp0;

    if (!fj) {
        if (mktemp(jname) < 0) {
            fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
15           cleanup(1);
        }
        if ((fj = fopen(jname, "w")) == 0) {
            fprintf(stderr, "%s: can't write %s\n", prog, jname);
            exit(1);
20       }
    }
    (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
    (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}
25

```

**Table 2**

5    PRO                        XXXXXXXXXXXXXXXXXX                         (Length = 15 amino acids)  
     Comparison Protein        XXXXXYYYYYYYYYY                         (Length = 12 amino acids)  
     % amino acid sequence identity =  
  
     (the number of identically matching amino acid residues between the two polypeptide sequences as  
 10   determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =  
       5 divided by 15 = 33.3%

**Table 3**

15    PRO                        XXXXXXXXXXXX                                 (Length = 10 amino acids)  
     Comparison Protein        XXXXXYYYYYYYZZYZ                         (Length = 15 amino acids)  
     % amino acid sequence identity =  
  
     (the number of identically matching amino acid residues between the two polypeptide sequences as  
 20   determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =  
       5 divided by 10 = 50%

**Table 4**

25    PRO-DNA                  NNNNNNNNNNNNNNN                         (Length = 14 nucleotides)  
     Comparison DNA            NNNNNNLLLLLLLLLLL                         (Length = 16 nucleotides)  
  
     % nucleic acid sequence identity =  
  
 30   (the number of identically matching nucleotides between the two nucleic acid sequences as determined by  
       ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =  
       6 divided by 14 = 42.9%

**Table 5**

35    PRO-DNA                  NNNNNNNNNNNNN                                 (Length = 12 nucleotides)  
     Comparison DNA            NNNNLLLVV                                     (Length = 9 nucleotides)  
  
     % nucleic acid sequence identity =

(the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =  
4 divided by 12 = 33.3%

5           II. Compositions and Methods of the Invention

A.       Full-Length PRO Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples 10 below. However, for sake of simplicity, in the present specification the protein encoded by the full length native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number", regardless of their origin or mode of preparation.

As disclosed in the Examples below, various cDNA clones have been disclosed. The predicted 15 amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

B.       PRO Polypeptide Variants

In addition to the full-length native sequence PRO polypeptides described herein, it is contemplated 20 that PRO variants can be prepared. PRO variants can be prepared by introducing appropriate nucleotide changes into the PRO DNA, and/or by synthesis of the desired PRO polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the PRO, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence PRO or in various domains of the PRO described 25 herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the PRO that results in a change in the amino acid sequence of the PRO as compared with the native sequence PRO. Optionally, the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the PRO. 30 Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the PRO with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, 35 i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

PRO polypeptide fragments are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native

protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the PRO polypeptide.

PRO fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating PRO fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, PRO polypeptide fragments share at least one biological and/or immunological activity with the native PRO polypeptide disclosed herein.

In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in reference to amino acid classes, are introduced and the products screened.

Table 6

	Original Residue	Exemplary Substitutions	Preferred Substitutions
20	Ala (A)	val; leu; ile	val
	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
25	Gln (Q)	asn	asn
	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe; norleucine	leu
30	Leu (L)	norleucine; ile; val; met; ala; phe	ile
	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
35	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
40	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in function or immunological identity of the PRO polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) hydrophobic: norleucine, met, ala, val, leu, ile;

- (2) neutral hydrophilic: cys, ser, thr;
  - (3) acidic: asp, glu;
  - (4) basic: asn, gln, his, lys, arg;
  - (5) residues that influence chain orientation: gly, pro; and
- 5      (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

10     The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., *Nucl. Acids Res.*, **13**:4331 (1986); Zoller et al., *Nucl. Acids Res.*, **10**:6487 (1987)], cassette mutagenesis [Wells et al., *Gene*, **34**:315 (1985)], restriction selection mutagenesis [Wells et al., *Philos. Trans. R. Soc. London SerA*, **317**:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the PRO variant DNA.

15     Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, **244**: 1081-1085 (1989)].  
20     Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, **150**:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

#### C. Modifications of PRO

25     Covalent modifications of PRO are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a PRO polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the PRO. Derivatization with bifunctional agents is useful, for instance, for crosslinking PRO to a water-insoluble support matrix or surface for use in the method for purifying anti-PRO antibodies, and vice-versa.  
30     Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[*p*-azidophenyl]dithio]propioimidate.

35     Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the PRO polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence PRO (either by removing the underlying glycosylation site or by deleting the 5 glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence PRO. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

10 Addition of glycosylation sites to the PRO polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence PRO (for O-linked glycosylation sites). The PRO amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the PRO polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

15 Another means of increasing the number of carbohydrate moieties on the PRO polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

20 Removal of carbohydrate moieties present on the PRO polypeptide may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 25 25 138:350 (1987).

Another type of covalent modification of PRO comprises linking the PRO polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

30 The PRO of the present invention may also be modified in a way to form a chimeric molecule comprising PRO fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the PRO with a tag 35 polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the PRO. The presence of such epitope-tagged forms of the PRO can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the PRO to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective 40 antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al.,

Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et al., Protein Engineering, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp et al., BioTechnology, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science, 255:192-194 (1992)]; an alpha-tubulin epitope peptide [Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the PRO with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The 10 Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a PRO polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

15      **D. Preparation of PRO**

The description below relates primarily to production of PRO by culturing cells transformed or transfected with a vector containing PRO nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare PRO. For instance, the PRO sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques 20 [see, e.g., Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the PRO may be chemically synthesized separately and combined using chemical or enzymatic 25 methods to produce the full-length PRO.

1.      **Isolation of DNA Encoding PRO**

DNA encoding PRO may be obtained from a cDNA library prepared from tissue believed to possess the PRO mRNA and to express it at a detectable level. Accordingly, human PRO DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. 30 The PRO-encoding gene may also be obtained from a genomic library or by known synthetic procedures (e.g., automated nucleic acid synthesis).

Libraries can be screened with probes (such as antibodies to the PRO or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as 35 described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding PRO is to use PCR methodology [Sambrook et al., *supra*; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide 40 sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives

are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like  $^{32}\text{P}$ -labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook et al., *supra*.

5 Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

10 Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook et al., *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

15 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for PRO production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue 20 experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in *Mammalian Cell Biotechnology: a Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook et al., *supra*.

Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example,  $\text{CaCl}_2$ ,  $\text{CaPO}_4$ , liposome-mediated and electroporation. Depending 25 on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., *supra*, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., *Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham 30 and van der Eb, *Virology*, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., *J. Bact.*, 130:946 (1977) and Hsiao et al., *Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or 35 polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., *Methods in Enzymology*, 185:527-537 (1990) and Mansour et al., *Nature*, 336:348-352 (1988).

Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as 40 Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli*

strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well  
5 as *Bacilli* such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation  
10 in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan'*; *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan'*; *E. coli* W3110 strain 40B4, which is strain  
15 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, e.g., PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for PRO-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower  
20 eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, Nature, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer et al., Bio/Technology, 9:968-975 (1991)) such as, e.g., *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., J. Bacteriol., 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae* (ATCC 36,906; Van  
25 den Berg et al., Bio/Technology, 8:135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., J. Basic Microbiol., 28:265-278 [1988]); *Candida*; *Trichoderma reesiae* (EP 244,234); *Neurospora crassa* (Case et al., Proc. Natl. Acad. Sci. USA, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357  
30 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., Biochem. Biophys. Res. Commun., 112:284-289 [1983]; Tilburn et al., Gene, 26:205-221 [1983]; Yelton et al., Proc. Natl. Acad. Sci. USA, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, EMBO J., 4:475-479 [1985]). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*,  
35 *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylotrophs, 269 (1982).

Suitable host cells for the expression of glycosylated PRO are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila S2* and *Spodoptera Sf9*, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and  
40 COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC

CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.*, 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse 5 mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (e.g., cDNA or genomic DNA) encoding PRO may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The 10 vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination 15 sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The PRO may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a 20 component of the vector, or it may be a part of the PRO-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, Ipp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* 25 glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to 30 replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable 35 marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the 40 identification of cells competent to take up the PRO-encoding nucleic acid, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR.

activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

5 Expression and cloning vectors usually contain a promoter operably linked to the PRO-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding PRO.

10 Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

15 Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytchrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 20 73,657.

25 PRO transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

30 Transcription of a DNA encoding the PRO by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from 35 mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the PRO coding sequence, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain 5 nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding PRO.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of PRO in recombinant vertebrate cell culture are described in Gething et al., *Nature*, 293:620-625 (1981); Mantei et al., *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

10           4.       Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be 15 employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as 20 immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence PRO polypeptide or against a 25 synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to PRO DNA and encoding a specific antibody epitope.

25           5.       Purification of Polypeptide

Forms of PRO may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or by enzymatic cleavage. Cells employed in expression of PRO can be disrupted by various physical or chemical 30 means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

It may be desired to purify PRO from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, 35 Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the PRO. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, *Methods in Enzymology*, 182 (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process 40 used and the particular PRO produced.

5            E.        Tissue Distribution

The location of tissues expressing the PRO can be identified by determining mRNA expression in various human tissues. The location of such genes provides information about which tissues are most likely to be affected by the stimulating and inhibiting activities of the PRO polypeptides. The location of a gene in a specific tissue also provides sample tissue for the activity blocking assays discussed below.

As noted before, gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal.

Conveniently, the antibodies may be prepared against a native sequence of a PRO polypeptide or against a synthetic peptide based on the DNA sequences encoding the PRO polypeptide or against an exogenous sequence fused to a DNA encoding a PRO polypeptide and encoding a specific antibody epitope. General techniques for generating antibodies, and special protocols for Northern blotting and *in situ* hybridization are provided below.

20            F.        Antibody Binding Studies

The activity of the PRO polypeptides can be further verified by antibody binding studies, in which the ability of anti-PRO antibodies to inhibit the effect of the PRO polypeptides, respectively, on tissue cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using

an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

5           G.     Cell-Based Assays

Cell-based assays and animal models for immune related diseases can be used to further understand the relationship between the genes and polypeptides identified herein and the development and pathogenesis of immune related disease.

In a different approach, cells of a cell type known to be involved in a particular immune related 10 disease are transfected with the cDNAs described herein, and the ability of these cDNAs to stimulate or inhibit immune function is analyzed. Suitable cells can be transfected with the desired gene, and monitored for immune function activity. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit or stimulate immune function, for example to modulate T-cell proliferation or inflammatory cell infiltration. Cells transfected with the coding sequences 15 of the genes identified herein can further be used to identify drug candidates for the treatment of immune related diseases.

In addition, primary cultures derived from transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell 20 lines from transgenic animals are well known in the art (see, e.g., Small *et al.*, *Mol. Cell. Biol.* 5: 642-648 [1985]).

One suitable cell based assay is the mixed lymphocyte reaction (MLR). *Current Protocols in Immunology*, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc. In this assay, the ability of a test compound to stimulate or inhibit the proliferation of activated T cells is assayed. A suspension of responder 25 T cells is cultured with allogeneic stimulator cells and the proliferation of T cells is measured by uptake of tritiated thymidine. This assay is a general measure of T cell reactivity. Since the majority of T cells respond to and produce IL-2 upon activation, differences in responsiveness in this assay in part reflect differences in IL-2 production by the responding cells. The MLR results can be verified by a standard lymphokine (IL-2) detection assay. *Current Protocols in Immunology*, above, 3.15, 6.3.

A proliferative T cell response in an MLR assay may be due to direct mitogenic properties of an assayed molecule or to external antigen induced activation. Additional verification of the T cell stimulatory 30 activity of the PRO polypeptides can be obtained by a costimulation assay. T cell activation requires an antigen specific signal mediated through the T-cell receptor (TCR) and a costimulatory signal mediated through a second ligand binding interaction, for example, the B7 (CD80, CD86)/CD28 binding interaction. CD28 crosslinking increases lymphokine secretion by activated T cells. T cell activation has both negative 35 and positive controls through the binding of ligands which have a negative or positive effect. CD28 and CTLA-4 are related glycoproteins in the Ig superfamily which bind to B7. CD28 binding to B7 has a positive costimulation effect of T cell activation; conversely, CTLA-4 binding to B7 has a T cell deactivating effect. Chambers, C. A. and Allison, J. P., *Curr. Opin. Immunol.* (1997) 9:396. Schwartz, R. 40 H., *Cell* (1992) 71:1065; Linsey, P. S. and Ledbetter, J. A., *Annu. Rev. Immunol.* (1993) 11:191; June, C. H.

*et al., Immunol. Today* (1994) 15:321; Jenkins, M. K., *Immunity* (1994) 1:405. In a costimulation assay, the PRO polypeptides are assayed for T cell costimulatory or inhibitory activity.

5 Direct use of a stimulating compound as in the invention has been validated in experiments with 4-1BB glycoprotein, a member of the tumor necrosis factor receptor family, which binds to a ligand (4-1BBL) expressed on primed T cells and signals T cell activation and growth. Alderson, M. E. *et al., J. Immunol.* (1994) 24:2219.

10 The use of an agonist stimulating compound has also been validated experimentally. Activation of 4-1BB by treatment with an agonist anti-4-1BB antibody enhances eradication of tumors. Hellstrom, I. and Hellstrom, K. E., *Crit. Rev. Immunol.* (1998) 18:1. Immunoadjuvant therapy for treatment of tumors, described in more detail below, is another example of the use of the stimulating compounds of the invention.

15 Alternatively, an immune stimulating or enhancing effect can also be achieved by administration of a PRO which has vascular permeability enhancing properties. Enhanced vascular permeability would be beneficial to disorders which can be attenuated by local infiltration of immune cells (*e.g.*, monocytes, eosinophils, PMNs) and inflammation.

20 On the other hand, PRO polypeptides, as well as other compounds of the invention, which are direct inhibitors of T cell proliferation/activation, lymphokine secretion, and/or vascular permeability can be directly used to suppress the immune response. These compounds are useful to reduce the degree of the immune response and to treat immune related diseases characterized by a hyperactive, superoptimal, or autoimmune response. This use of the compounds of the invention has been validated by the experiments described above in which CTLA-4 binding to receptor B7 deactivates T cells. The direct inhibitory compounds of the invention function in an analogous manner. The use of compound which suppress vascular permeability would be expected to reduce inflammation. Such uses would be beneficial in treating conditions associated with excessive inflammation.

25 Alternatively, compounds, *e.g.*, antibodies, which bind to stimulating PRO polypeptides and block the stimulating effect of these molecules produce a net inhibitory effect and can be used to suppress the T cell mediated immune response by inhibiting T cell proliferation/activation and/or lymphokine secretion. Blocking the stimulating effect of the polypeptides suppresses the immune response of the mammal. This use has been validated in experiments using an anti-IL2 antibody. In these experiments, the antibody binds 30 to IL2 and blocks binding of IL2 to its receptor thereby achieving a T cell inhibitory effect.

#### H. Animal Models

35 The results of the cell based *in vitro* assays can be further verified using *in vivo* animal models and assays for T-cell function. A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of immune related disease, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them predictive of responses in human patients. Animal models of immune related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, *e.g.*, murine models. Such models can be generated by introducing cells into syngeneic mice using

standard techniques, *e.g.*, subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, *etc.*

Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life threatening severe inflammation to mild cases of diarrhea and weight loss. Graft-versus-host disease models provide a means of assessing T cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.3.

An animal model for skin allograft rejection is a means of testing the ability of T cells to mediate *in vivo* tissue destruction and a measure of their role in transplant rejection. The most common and accepted models use murine tail-skin grafts. Repeated experiments have shown that skin allograft rejection is mediated by T cells, helper T cells and killer-effector T cells, and not antibodies. Auchincloss, H. Jr. and Sachs, D. H., *Fundamental Immunology*, 2nd ed., W. E. Paul ed., Raven Press, NY, 1989, 889-992. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.4. Other transplant rejection models which can be used to test the compounds of the invention are the allogeneic heart transplant models described by Tanabe, M. *et al*, *Transplantation* (1994) 58:23 and Tinubu, S. A. *et al*, *J. Immunol.* (1994) 4330-4338.

Animal models for delayed type hypersensitivity provides an assay of cell mediated immune function as well. Delayed type hypersensitivity reactions are a T cell mediated *in vivo* immune response characterized by inflammation which does not reach a peak until after a period of time has elapsed after challenge with an antigen. These reactions also occur in tissue specific autoimmune diseases such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE, a model for MS). A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.5.

EAE is a T cell mediated autoimmune disease characterized by T cell and mononuclear cell inflammation and subsequent demyelination of axons in the central nervous system. EAE is generally considered to be a relevant animal model for MS in humans. Bolton, C., *Multiple Sclerosis* (1995) 1:143. Both acute and relapsing-remitting models have been developed. The compounds of the invention can be tested for T cell stimulatory or inhibitory activity against immune mediated demyelinating disease using the protocol described in *Current Protocols in Immunology*, above, units 15.1 and 15.2. See also the models for myelin disease in which oligodendrocytes or Schwann cells are grafted into the central nervous system as described in Duncan, I. D. *et al*, *Molec. Med. Today* (1997) 554-561.

Contact hypersensitivity is a simple delayed type hypersensitivity *in vivo* assay of cell mediated immune function. In this procedure, cutaneous exposure to exogenous haptens which gives rise to a delayed type hypersensitivity reaction which is measured and quantitated. Contact sensitivity involves an initial sensitizing phase followed by an elicitation phase. The elicitation phase occurs when the T lymphocytes encounter an antigen to which they have had previous contact. Swelling and inflammation occur, making this an excellent model of human allergic contact dermatitis. A suitable procedure is described in detail in *Current Protocols in Immunology*, Eds. J. E. Cologan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, John Wiley & Sons, Inc., 1994, unit 4.2. See also Grabbe, S. and Schwarz, T, *Immun. Today* 19 (1): 37-44 (1998).

An animal model for arthritis is collagen-induced arthritis. This model shares clinical, histological and immunological characteristics of human autoimmune rheumatoid arthritis and is an acceptable model for human autoimmune arthritis. Mouse and rat models are characterized by synovitis, erosion of cartilage and subchondral bone. The compounds of the invention can be tested for activity against autoimmune arthritis  
5 using the protocols described in *Current Protocols in Immunology*, above, units 15.5. See also the model using a monoclonal antibody to CD18 and VLA-4 integrins described in Issekutz, A.C. et al., *Immunology* (1996) 88:569.

A model of asthma has been described in which antigen-induced airway hyper-reactivity, pulmonary eosinophilia and inflammation are induced by sensitizing an animal with ovalbumin and then  
10 challenging the animal with the same protein delivered by aerosol. Several animal models (guinea pig, rat, non-human primate) show symptoms similar to atopic asthma in humans upon challenge with aerosol antigens. Murine models have many of the features of human asthma. Suitable procedures to test the compounds of the invention for activity and effectiveness in the treatment of asthma are described by Wolyniec, W. W. et al, *Am. J. Respir. Cell Mol. Biol.* (1998) 18:777 and the references cited therein.

15 Additionally, the compounds of the invention can be tested on animal models for psoriasis like diseases. Evidence suggests a T cell pathogenesis for psoriasis. The compounds of the invention can be tested in the scid/scid mouse model described by Schon, M. P. et al, *Nat. Med.* (1997) 3:183, in which the mice demonstrate histopathologic skin lesions resembling psoriasis. Another suitable model is the human skin/scid mouse chimera prepared as described by Nickoloff, B. J. et al, *Am. J. Path.* (1995) 146:580.

20 Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, e.g., baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include  
25 pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines (e.g., Van der Putten et al., *Proc. Natl. Acad. Sci. USA* 82, 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson et al., *Cell* 56, 313-321 [1989]); electroporation of embryos (*Lo, Mol. Cel. Biol.* 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano et al., *Cell* 57, 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

30 For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, e.g., head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko et al., *Proc. Natl. Acad. Sci. USA* 89, 6232-636 (1992).

35 The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry.

40 The animals may be further examined for signs of immune disease pathology, for example by histological examination to determine infiltration of immune cells into specific tissues. Blocking

experiments can also be performed in which the transgenic animals are treated with the compounds of the invention to determine the extent of the T cell proliferation stimulation or inhibition of the compounds. In these experiments, blocking antibodies which bind to the PRO polypeptide, prepared as described above, are administered to the animal and the effect on immune function is determined.

5        Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular polypeptide can be used to clone 10 genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA 15 has homologously recombined with the endogenous DNA are selected [see e.g., Li *et al.*, *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a 20 "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the polypeptide.

25        I.        ImmunoAdjuvant Therapy

In one embodiment, the immunostimulating compounds of the invention can be used in immunoadjuvant therapy for the treatment of tumors (cancer). It is now well established that T cells recognize human tumor specific antigens. One group of tumor antigens, encoded by the MAGE, BAGE and GAGE families of genes, are silent in all adult normal tissues, but are expressed in significant amounts in 30 tumors, such as melanomas, lung tumors, head and neck tumors, and bladder carcinomas. DeSmet, C. *et al.*, (1996) *Proc. Natl. Acad. Sci. USA*, 93:7149. It has been shown that costimulation of T cells induces tumor regression and an antitumor response both *in vitro* and *in vivo*. Melero, I. *et al.*, *Nature Medicine* (1997) 3:682; Kwon, E. D. *et al.*, *Proc. Natl. Acad. Sci. USA* (1997) 94: 8099; Lynch, D. H. *et al.*, *Nature Medicine* (1997) 3:625; Finn, O. J. and Lotze, M. T., *J. Immunol.* (1998) 21:114. The stimulatory compounds of the 35 invention can be administered as adjuvants, alone or together with a growth regulating agent, cytotoxic agent or chemotherapeutic agent, to stimulate T cell proliferation/activation and an antitumor response to tumor antigens. The growth regulating, cytotoxic, or chemotherapeutic agent may be administered in conventional amounts using known administration regimes. Immunostimulating activity by the compounds of the invention allows reduced amounts of the growth regulating, cytotoxic, or chemotherapeutic agents thereby 40 potentially lowering the toxicity to the patient.

J. Screening Assays for Drug Candidates

Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the genes identified herein or a biologically active fragment thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art. All assays are common in that they call for contacting the drug candidate with a polypeptide encoded by a nucleic acid identified herein under conditions and for a time sufficient to allow these two components to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.*, on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, *e.g.*, a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.*, the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.*, by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular protein encoded by a gene identified herein, its interaction with that protein can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature (London)* **340**, 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* **88**, 9578-9582 (1991)] as disclosed by Chevray and Nathans, *Proc. Natl. Acad. Sci. USA* **89**, 5789-5793 (1991). Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate

activating proteins are fused to the activation domain. The expression of a GAL1-lacZ reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER<sup>TM</sup>) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

In order to find compounds that interfere with the interaction of a gene identified herein and other intra- or extracellular components can be tested, a reaction mixture is usually prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the two products. To test the ability of a test compound to inhibit binding, the reaction is run in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described above. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

#### K. Compositions and Methods for the Treatment of Immune Related Diseases

The compositions useful in the treatment of immune related diseases include, without limitation, proteins, antibodies, small organic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, etc. that inhibit or stimulate immune function, for example, T cell proliferation/activation, lymphokine release, or immune cell infiltration.

For example, antisense RNA and RNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, e.g., Rossi, *Current Biology* 4, 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, e.g., PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any or any combination of the screening assays discussed above and/or by any other screening techniques well known for those skilled in the art.

5           L.        Anti-PRO Antibodies

The present invention further provides anti-PRO antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies.

10          1.        Polyclonal Antibodies

The anti-PRO antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections.

15          The immunizing agent may include the PRO polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

20          2.        Monoclonal Antibodies

The anti-PRO antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

25          The immunizing agent will typically include the PRO polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

30          Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described 35 for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al.,

Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against PRO. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin.

5 Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by

10 corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The

15 humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., J. Immunol., 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous

35 immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology 10, 779-783 (1992); Lonberg et al., Nature 368 856-859 (1994);

Morrison, Nature **368**, 812-13 (1994); Fishwild *et al.*, Nature Biotechnology **14**, 845-51 (1996); Neuberger, Nature Biotechnology **14**, 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol. **13** 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 5 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

#### 4. Bispecific Antibodies

10 Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the PRO, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

15 Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities [Milstein and Cuello, Nature, **305**:537-539 (1983)]. Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, 20 and in Traunecker *et al.*, EMBO J., **10**:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions.

It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, **121**:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g. tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g. alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

40 Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared can be prepared using

chemical linkage. Brennan *et al.*, *Science* 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of 5 the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized 10 bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various technique for making and isolating bispecific antibody fragments directly from 15 recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form 20 the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby 25 forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber *et al.*, *J. Immunol.* 152:5368 (1994). Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given PRO polypeptide 30 herein. Alternatively, an anti-PRO polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular PRO polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular PRO polypeptide. These antibodies 35 possess a PRO-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the PRO polypeptide and further binds tissue factor (TF).

##### 5. Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate 40 antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been

proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptopbutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

#### 6. Effector Function Engineering

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) may be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, J. Exp Med., 176: 1191-1195 (1992) and Shope, J. Immunol., 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* Cancer Research, 53: 2560-2565 (1993). Alternatively, an antibody can be engineered that has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, Anti-Cancer Drug Design, 3: 219-230 (1989).

#### 7. Immunoconjugates

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the trichothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include <sup>212</sup>Bi, <sup>131</sup>I, <sup>131</sup>In, <sup>90</sup>Y, and <sup>186</sup>Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridylthiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis-(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) that is conjugated to a cytotoxic agent (e.g., a radionucleotide).

5           8.       Immunoliposomes

The antibodies disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang *et al.*, Proc. Natl. Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent 10 No. 5,013,556.

Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be 15 conjugated to the liposomes as described in Martin *et al.*, J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al.*, J. National Cancer Inst., 81(19): 1484 (1989).

M.       Pharmaceutical Compositions

The active PRO molecules of the invention (e.g., PRO polypeptides, anti-PRO antibodies, and/or variants of each) as well as other molecules identified by the screening assays disclosed above, can be administered for the treatment of immune related diseases, in the form of pharmaceutical compositions.

Therapeutic formulations of the active PRO molecule, preferably a polypeptide or antibody of the invention, are prepared for storage by mixing the active molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; 25 alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as 30 sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONICS<sup>TM</sup> or polyethylene glycol (PEG).

Compounds identified by the screening assays disclosed herein can be formulated in an analogous manner, using standard techniques well known in the art.

Lipofections or liposomes can also be used to deliver the PRO molecule into cells. Where antibody fragments are used, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be 5 synthesized chemically and/or produced by recombinant DNA technology (see, e.g., Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* **90**, 7889-7893 [1993]).

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or 10 growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active PRO molecules may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems 15 (for example, liposomes, albumin microspheres, macroemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations or the PRO molecules may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$ -ethyl-L- 20 glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins 25 for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S 30 bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulphydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

#### N. Methods of Treatment

It is contemplated that the polypeptides, antibodies and other active compounds of the present invention may be used to treat various immune related diseases and conditions, such as T cell mediated diseases, including those characterized by infiltration of inflammatory cells into a tissue, stimulation of T-

cell proliferation, inhibition of T-cell proliferation, increased or decreased vascular permeability or the inhibition thereof.

Exemplary conditions or disorders to be treated with the polypeptides, antibodies and other compounds of the invention, include, but are not limited to systemic lupus erythematosis, rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft -versus-host-disease.

In systemic lupus erythematosus, the central mediator of disease is the production of auto-reactive antibodies to self proteins/tissues and the subsequent generation of immune-mediated inflammation. Antibodies either directly or indirectly mediate tissue injury. Though T lymphocytes have not been shown to be directly involved in tissue damage, T lymphocytes are required for the development of auto-reactive antibodies. The genesis of the disease is thus T lymphocyte dependent. Multiple organs and systems are affected clinically including kidney, lung, musculoskeletal system, mucocutaneous, eye, central nervous system, cardiovascular system, gastrointestinal tract, bone marrow and blood.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes; the joint space/fluid if infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs in two major forms. One form is the development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin

ulcers and gangrene. Patients often also develop rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations which can occur in RA include: pericarditis, pleuritis, coronary arteritis, interstitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rheumatoid nodules.

5 Juvenile chronic arthritis is a chronic idiopathic inflammatory disease which begins often at less than 16 years of age. Its phenotype has some similarities to RA; some patients which are rheumatoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic  
10 anterior uveitis and systemic amyloidosis.

Spondyloarthropathies are a group of disorders with some common clinical features and the common association with the expression of HLA-B27 gene product. The disorders include: ankylosing spondylitis, Reiter's syndrome (reactive arthritis), arthritis associated with inflammatory bowel disease, spondylitis associated with psoriasis, juvenile onset spondyloarthropathy and undifferentiated  
15 spondyloarthropathy. Distinguishing features include sacroileitis with or without spondylitis; inflammatory asymmetric arthritis; association with HLA-B27 (a serologically defined allele of the HLA-B locus of class I MHC); ocular inflammation, and absence of autoantibodies associated with other rheumatoid disease. The cell most implicated as key to induction of the disease is the CD8+ T lymphocyte, a cell which targets antigen presented by class I MHC molecules. CD8+ T cells may react against the class I MHC allele HLA-B27 as if it were a foreign peptide expressed by MHC class I molecules. It has been hypothesized that an  
20 epitope of HLA-B27 may mimic a bacterial or other microbial antigenic epitope and thus induce a CD8+ T cells response.

Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is induced by an active inflammatory process. Scleroderma can be localized or  
25 systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An immunologic basis is implied by the presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin lesions suggesting that T cell interaction with these cells may have a role in the  
30 pathogenesis of the disease. Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis,  
35 scarring/fibrosis.

Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-specific autoantibodies are directed against and inhibit the function of components, proteins  
40 and RNA's, involved in protein synthesis.

Sjögren's syndrome is due to immune-mediated inflammation and subsequent functional destruction of the tear glands and salivary glands. The disease can be associated with or accompanied by inflammatory connective tissue diseases. The disease is associated with autoantibody production against Ro and La antigens, both of which are small RNA-protein complexes. Lesions result in keratoconjunctivitis sicca, 5 xerostomia, with other manifestations or associations including biliary cirrhosis, peripheral or sensory neuropathy, and palpable purpura.

Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by the affected vessels and eventual end-organ dysfunction in some cases. Vasculitides can also occur as a secondary lesion 10 or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, etc., particularly in diseases also associated with the formation of immune complexes. Diseases in the primary systemic vasculitis group include: systemic necrotizing vasculitis: polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis. Miscellaneous vasculitides include: mucocutaneous lymph node syndrome (MLNS or 15 Kawasaki's disease), isolated CNS vasculitis, Behet's disease, thromboangiitis obliterans (Buerger's disease) and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or both.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid 20 granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, 25 and paroxysmal nocturnal hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated 30 thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and 35 atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

Type I diabetes mellitus or insulin-dependent diabetes is the autoimmune destruction of pancreatic islet  $\beta$  cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are 5 the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition of antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune 10 mechanisms result in inflammatory response that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

Demyelinating diseases of the central and peripheral nervous systems, including Multiple Sclerosis; idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome; and Chronic Inflammatory 15 Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is evidence to suggest that disease induction and progression is dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition, environment, and 20 autoimmunity all contribute. Lesions contain infiltrates of predominantly T lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+ T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely T lymphocyte driven.

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic 25 Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a disregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

30 Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Allergic diseases, including asthma; allergic rhinitis; atopic dermatitis; food hypersensitivity; and urticaria are T lymphocyte dependent. These diseases are predominantly mediated by T lymphocyte induced inflammation, IgE mediated-inflammation or a combination of both.

35 Transplantation associated diseases, including Graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E and herpes) bacterial infection, fungal infections, and protozoal and parasitic infections

40 (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the

immune response to infectious agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (*i.e.*, as from chemotherapy) immunodeficiency, and neoplasia.

5 It has been demonstrated that some human cancer patients develop an antibody and/or T lymphocyte response to antigens on neoplastic cells. It has also been shown in animal models of neoplasia that enhancement of the immune response can result in rejection or regression of that particular neoplasm. Molecules that enhance the T lymphocyte response in the MLR have utility *in vivo* in enhancing the immune response against neoplasia. Molecules which enhance the T lymphocyte proliferative response in the MLR  
10 (or small molecule agonists or antibodies that affected the same receptor in an agonistic fashion) can be used therapeutically to treat cancer. Molecules that inhibit the lymphocyte response in the MLR also function *in vivo* during neoplasia to suppress the immune response to a neoplasm; such molecules can either be expressed by the neoplastic cells themselves or their expression can be induced by the neoplasm in other cells. Antagonism of such inhibitory molecules (either with antibody, small molecule antagonists or other  
15 means) enhances immune-mediated tumor rejection.

Additionally, inhibition of molecules with proinflammatory properties may have therapeutic benefit in reperfusion injury; stroke; myocardial infarction; atherosclerosis; acute lung injury; hemorrhagic shock; burn; sepsis/septic shock; acute tubular necrosis; endometriosis; degenerative joint disease and pancreatitis.

20 The compounds of the present invention, *e.g.*, polypeptides or antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation (intranasal, intrapulmonary) routes. Intravenous or inhaled administration of polypeptides and antibodies is preferred.

25 In immunoadjuvant therapy, other therapeutic regimens, such administration of an anti-cancer agent, may be combined with the administration of the proteins, antibodies or compounds of the instant invention. For example, the patient to be treated with the immunoadjuvant of the invention may also receive an anti-cancer agent (chemotherapeutic agent) or radiation therapy. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy  
30 are also described in *Cancer Treatment Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the immunoadjuvant or may be given simultaneously therewith. Additionally, an anti-estrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) may be given in dosages known for such molecules.

35 It may be desirable to also administer antibodies against other immune disease associated or tumor associated antigens, such as antibodies which bind to CD20, CD11a, CD18, ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be coadministered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. In one embodiment, the PRO polypeptides are coadministered with a growth inhibitory agent. For example, the growth inhibitory agent  
40 may be administered first, followed by a PRO polypeptide. However, simultaneous administration or

administration first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and the PRO polypeptide.

For the treatment or reduction in the severity of immune related disease, the appropriate dosage of 5 an a compound of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

10 For example, depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g., 0.1-20 mg/kg) of polypeptide or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is 15 sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

O. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials (e.g., comprising a PRO molecule) useful for the diagnosis or treatment of the disorders described above is 20 provided. The article of manufacture comprises a container and an instruction. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the 25 composition is usually a polypeptide or an antibody of the invention. An instruction or label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including 30 other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

P. Diagnosis and Prognosis of Immune Related Disease

Cell surface proteins, such as proteins which are overexpressed in certain immune related diseases, are excellent targets for drug candidates or disease treatment. The same proteins along with secreted 35 proteins encoded by the genes amplified in immune related disease states find additional use in the diagnosis and prognosis of these diseases. For example, antibodies directed against the protein products of genes amplified in multiple sclerosis, rheumatoid arthritis, or another immune related disease, can be used as diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used to qualitatively or 40 quantitatively detect the expression of proteins encoded by amplified or overexpressed genes ("marker gene products"). The antibody preferably is equipped with a detectable, e.g., fluorescent label, and binding can be

monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable, if the overexpressed gene encodes a cell surface protein. Such binding assays are performed essentially as described above.

5 *In situ* detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

10 The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

#### EXAMPLES

15 Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA.

20 **EXAMPLE 1: Microarray analysis of stimulated T-cells**

Nucleic acid microarrays, often containing thousands of gene sequences, are useful for identifying differentially expressed genes in diseased tissues as compared to their normal counterparts. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The cDNA probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes known to be expressed in certain disease states may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene. If the hybridization signal of a probe from a test (in this instance, activated CD4+ T cells) sample is greater than the hybridization signal of a probe from a control (in this instance, non-stimulated CD4 + T cells) sample, the gene or genes overexpressed in the test tissue are identified. The implication of this result is that an overexpressed protein in a test tissue is useful not only as a diagnostic marker for the presence of the disease condition, but also as a therapeutic target for treatment of the disease condition.

35 The methodology of hybridization of nucleic acids and microarray technology is well known in the art. In one example, the specific preparation of nucleic acids for hybridization and probes, slides, and hybridization conditions are all detailed in PCT Patent Application Serial No. PCT/US01/10482, filed on March 30, 2001 and which is herein incorporated by reference.

40 In this experiment, CD4+ T cells were purified from a single donor using the RosetteSep™ protocol from (Stem Cell Technologies, Vancouver BC) which contains anti-CD8, anti-CD16, anti-CD19, anti-CD36 and anti-CD56 antibodies used to produce a population of isolated CD4 + T cells. Isolated CD4+

T cells were activated with an anti-CD3 antibody (used at a concentration that does not stimulate proliferation) together with either ICAM-1 or anti-CD28 antibody. At 24 or 72 hours cells were harvested, RNA extracted and analysis run on Affimax (Affymetrix Inc. Santa Clara, CA) microarray chips. Non-stimulated (resting) cells were harvested immediately after purification, and subjected to the same analysis.

5 Genes were compared whose expression was upregulated at either of the two timepoints in activated vs. resting cells.

Below are the results of these experiments, demonstrating that various PRO polypeptides of the present invention are differentially expressed in isolated CD4 + T cells activated by anti-CD3/ICAM-1 or anti-CD3/anti-CD28 as compared to isolated resting CD4+ T cells. As described above, these data 10 demonstrate that the PRO polypeptides of the present invention are useful not only as diagnostic markers for the presence of one or more immune disorders, but also serve as therapeutic targets for the treatment of those immune disorders.

The results of this experiment are Figures 1-7589 show increase or decrease in expression upon stimulation with anti-CD3/ICAM1 and also show increase or decrease in expression upon stimulation with 15 anti-CD3/anti-CD28. The nucleic acids and encoded proteins of Figure 946, Figure 1520, Figure 1574, Figure 1622, Figure 1816, Figure 2433, Figure 2986, Figure 3220, Figure 4120 and Figure 5421 are significantly overexpressed in isolated CD4 + T cells activated by anti-CD3/ICAM-1 or anti-CD3/anti-CD28 as compared to isolated resting CD4+ T cells.

20 **EXAMPLE 2: Use of PRO as a hybridization probe**

The following method describes use of a nucleotide sequence encoding PRO as a hybridization probe.

DNA comprising the coding sequence of full-length or mature PRO as disclosed herein is employed 25 as a probe to screen for homologous DNAs (such as those encoding naturally-occurring variants of PRO) in human tissue cDNA libraries or human tissue genomic libraries.

Hybridization and washing of filters containing either library DNAs is performed under the following high stringency conditions. Hybridization of radiolabeled PRO-derived probe to the filters is performed in a solution of 50% formamide, 5x SSC, 0.1% SDS, 0.1% sodium pyrophosphate, 50 mM sodium phosphate, pH 6.8, 2x Denhardt's solution, and 10% dextran sulfate at 42°C for 20 hours. Washing 30 of the filters is performed in an aqueous solution of 0.1x SSC and 0.1% SDS at 42°C.

DNAs having a desired sequence identity with the DNA encoding full-length native sequence PRO can then be identified using standard techniques known in the art.

**EXAMPLE 3: Expression of PRO in *E. coli***

35 This example illustrates preparation of an unglycosylated form of PRO by recombinant expression in *E. coli*.

The DNA sequence encoding PRO is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is 40 pBR322 (derived from *E. coli*; see Bolivar et al., *Gene*, 2:95 (1977)) which contains genes for ampicillin and

tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the PRO coding region, lambda transcriptional terminator, and an argU gene.

5 The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook et al., *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

10 Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

15 After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized PRO protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein.

20 PRO may be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding PRO is initially amplified using selected PCR primers. The primers will contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then ligated into an expression vector, which is used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq)). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are 25 then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.71 g sodium citrate•2H<sub>2</sub>O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO<sub>4</sub>) and grown for approximately 20-30 hours at 30°C with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

30 *E. coli* paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of 35 metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. The clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with buffer containing 250 mM imidazole. Fractions containing the desired protein are pooled and stored at 4°C. Protein concentration is estimated by 40 its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting the sample slowly into freshly prepared refolding buffer consisting of: 20 mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4°C for 12-36 hours. The refolding reaction is  
5 quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions  
10 containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

15 Fractions containing the desired folded PRO polypeptide are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.  
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#### EXAMPLE 4: Expression of PRO in mammalian cells

This example illustrates preparation of a potentially glycosylated form of PRO by recombinant expression in mammalian cells.

25 The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the PRO DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the PRO DNA using ligation methods such as described in Sambrook et al., *supra*. The resulting vector is called pRK5-PRO.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-PRO DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya et al., *Cell*, 31:543 (1982)] and dissolved in 500 µl of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl<sub>2</sub>. To this mixture is added, dropwise, 500 µl of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO<sub>4</sub>, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at  
30 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

35 Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml <sup>35</sup>S-cysteine and 200 µCi/ml <sup>35</sup>S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter,  
40

and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of PRO polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and the medium is tested in selected bioassays.

In an alternative technique, PRO may be introduced into 293 cells transiently using the dextran sulfate method described by Somparyrac et al., *Proc. Natl. Acad. Sci.*, 78:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-PRO DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed PRO can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, PRO can be expressed in CHO cells. The pRK5-PRO can be transfected into CHO cells using known reagents such as CaPO<sub>4</sub> or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as <sup>35</sup>S-methionine. After determining the presence of PRO polypeptide, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed PRO can then be concentrated and purified by any selected method.

Epitope-tagged PRO may also be expressed in host CHO cells. The PRO may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged PRO insert can then be subcloned into a SV40 promoter/enhancer containing vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 promoter/enhancer containing vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged PRO can then be concentrated and purified by any selected method, such as by Ni<sup>2+</sup>-chelate affinity chromatography.

PRO may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

Stable expression in CHO cells is performed using the following procedure. The proteins are expressed as an IgG construct (immunoadhesin), in which the coding sequences for the soluble forms (e.g. extracellular domains) of the respective proteins are fused to an IgG1 constant region sequence containing the hinge, CH2 and CH2 domains and/or is a poly-His tagged form.

Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel et al., *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNA's. The vector used expression in CHO cells is as described in Lucas et al., *Nucl. Acids Res.*, 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect® (Qiagen), Dospex® or Fugene® (Boehringer Mannheim). The cells are grown as described in Lucas et al., *supra*. Approximately  $3 \times 10^7$  cells are frozen in an ampule for further growth and production as described below.

5       The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by vortexing. The contents are pipetted into a centrifuge tube containing 10 mL of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2 µm filtered PS20 with 5% 0.2 µm diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a  
10 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with  $3 \times 10^5$  cells/mL. The cell media is exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at  $1.2 \times 10^6$  cells/mL. On day 0, pH is determined. On  
15 day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22 µm filter.  
20      The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column  
25 is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalting into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The  
30 conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 µl of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalting into storage buffer as described above for the poly-His tagged proteins. The  
35 homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

**EXAMPLE 5: Expression of PRO in Yeast**

40      The following method describes recombinant expression of PRO in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of PRO from the ADH2/GAPDH promoter. DNA encoding PRO and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of PRO. For secretion, DNA encoding PRO can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native PRO signal peptide or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of PRO.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining 10 of the gels with Coomassie Blue stain.

Recombinant PRO can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing PRO may further be purified using selected column chromatography resins.

Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

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#### EXAMPLE 6: Expression of PRO in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of PRO in Baculovirus-infected insect cells.

The sequence coding for PRO is fused upstream of an epitope tag contained within a baculovirus 20 expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding PRO or the desired portion of the coding sequence of PRO such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular is amplified by PCR with primers 25 complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfected the above plasmid and BaculoGold<sup>TM</sup> virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin 30 (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994).

Expressed poly-his tagged PRO can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity 35 chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert et al., Nature, 362:175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered 40 through a 0.45 µm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared

with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and silver staining or Western blot with Ni<sup>2+</sup>-NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged PRO are pooled and dialyzed against loading buffer.

5 Alternatively, purification of the IgG tagged (or Fc tagged) PRO can be performed using known chromatography techniques, including for instance, Protein A or protein G column chromatography.

10 Many of the PRO polypeptides disclosed herein were successfully expressed as described above.

**EXAMPLE 7: Preparation of Antibodies that Bind PRO**

This example illustrates preparation of monoclonal antibodies which can specifically bind PRO.

15 Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified PRO, fusion proteins containing PRO, and cells expressing recombinant PRO on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

20 Mice, such as Balb/c, are immunized with the PRO immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms.

Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-PRO antibodies.

25 After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of PRO. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597. The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

30 The hybridoma cells will be screened in an ELISA for reactivity against PRO. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against PRO is within the skill in the art.

35 The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-PRO monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion

chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

**EXAMPLE 8: Purification of PRO Polypeptides Using Specific Antibodies**

5 Native or recombinant PRO polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-PRO polypeptide, mature PRO polypeptide, or pre-PRO polypeptide is purified by immunoaffinity chromatography using antibodies specific for the PRO polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-PRO polypeptide antibody to an activated chromatographic resin.

10 Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE<sup>TM</sup> (Pharmacia LKB 15 Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of PRO polypeptide by preparing a fraction from cells containing PRO polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the 20 addition of detergent or by other methods well known in the art. Alternatively, soluble PRO polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble PRO polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRO polypeptide (*e.g.*, high 25 ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/PRO polypeptide binding (*e.g.*, a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotropic such as urea or thiocyanate ion), and PRO polypeptide is collected.

**EXAMPLE 9: Drug Screening**

30 This invention is particularly useful for screening compounds by using PRO polypeptides or binding fragment thereof in any of a variety of drug screening techniques. The PRO polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the PRO polypeptide or fragment. 35 Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between PRO polypeptide or a fragment and the agent being tested. Alternatively, one can examine the diminution in complex formation between the PRO polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a PRO polypeptide-associated disease or disorder. These methods comprise contacting such an agent with an PRO polypeptide or fragment thereof and assaying (I) for the presence of a complex between the agent and the PRO polypeptide or fragment, or (ii) for the presence of a complex between the PRO polypeptide or fragment and the cell, by methods well known in the art. In such competitive binding assays, the PRO polypeptide or fragment is typically labeled. After suitable incubation, free PRO polypeptide or fragment is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to PRO polypeptide or to interfere with the PRO polypeptide/cell complex.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a PRO polypeptide, the peptide test compounds are reacted with PRO polypeptide and washed. Bound PRO polypeptide is detected by methods well known in the art. Purified PRO polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding PRO polypeptide specifically compete with a test compound for binding to PRO polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRO polypeptide.

#### EXAMPLE 10: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a PRO polypeptide) or of small molecules with which they interact, *e.g.*, agonists, antagonists, or inhibitors. Any of these examples can be used to fashion drugs which are more active or stable forms of the PRO polypeptide or which enhance or interfere with the function of the PRO polypeptide *in vivo* (*c.f.*, Hodgson, Bio/Technology, 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the PRO polypeptide, or of a PRO polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the PRO polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the PRO polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous PRO polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton and Wells, Biochemistry, 31:7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, J. Biochem., 113:742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which

subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or 5 biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the PRO polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the PRO polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

10 The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable 15 the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustrations that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.